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THE NEURONAL CONTROL OF THE ANTENNULAR ACTIVITIES OF THE HERMIT CRAB, *PAGURUS ALASKENSIS* (BENEDICT)

by

© PETER JOHN SNOW

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE NEURONAL CONTROL OF THE ANTENNULAR ACTIVITIES OF THE HERMIT CRAB, *PAGURUS ALASKENSIS* (BENEDICT), submitted by Peter John Snow in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The surface structures of the antennular flagella of the hermit crab, *Pagurus alaskensis* (Benedict) are described in detail. Attention is directed towards the surface morphology of possible sensilla. These may be divided into two major categories: (1) exoskeletal pores (1.0 to 3.0 μm in diameter) and (2) a variety of types of setae. It is suggested that all the setae serve a sensory function. The functions of the various setae are discussed in relation to their topographical location and to existing electrophysiological and behavioural data.

The antennular activities have been described with the aid of motion pictures. Four types of activity may be defined: flicking, rotation, wiping and withdrawal. It is suggested that flicking facilitates the chemoreceptive process by exchanging the water trapped around the densely packed chemoreceptive aesthetasc setae. Antennular rotation could aid this process by ensuring that most flicks are directed into water currents. Wiping serves to remove debris caught amongst the aesthetascs while antennular withdrawal probably serves to remove the antennules from potentially noxious stimuli.

The motor innervation and musculature of the medial and distal segments of the antennule have been described anatomically. Intracellular recordings within these muscles and simultaneous monitoring of whole-muscle tension have been used to define the motoneurons and contractile properties of the muscle fibres they innervate. The antennular motor system may be divided into three components: a phasic component (motor units 30F, 31F and 32F), a tonic component (motor units 30S, 31S and 32S) and a phaso-tonic component (motor unit 31F-S).

Using electromyogram recording from the muscles of the medial and distal segments, the patterns of activity in specific antennular motoneurons have been described during antennular flicking and withdrawal. Only the phasic component of the motor system, motoneurons A30F, A31F and A32F, is active during flicking. The functional significance of activity patterns in these motoneurons is discussed with reference to the proposed function of flicking. Extension-withdrawal and slow flexion-withdrawal reflexes, tonic flexion withdrawal and maintained flexion at the medial segment-distal segment joint result from activity in the tonic component of the motor system: motoneurons A30S, A31S and A32S. The patterning of activity in these motoneurons is discussed. Fast flexion-withdrawal reflexes result from a burst of spikes in motoneuron A31F-S which constitutes the phaso-tonic component of the antennular motor system. The functional significance of this motoneuronal activity is discussed in relation to the anatomy and physiology of motor unit 31F-S and the form and speed of fast flexion-withdrawal reflexes.

The effects of altering sensory input on the motoneuronal patterns underlying antennular flicking have been tested. The results are incorporated into a model in which flexor activity is triggered while extensor activity is reflexively elicited by feedback from receptors sensitive to joint flexion. The functional significance of reflex control of extensor activity is discussed in relation to both the form and the proposed function of antennular flicking and to the general role of reflexes in stereotyped activities of other invertebrates.

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I. INTRODUCTION

In the last fifteen years the nervous origin of stereotyped activities has been a major area of neurophysiological research (see reviews: Bullock, 1961; DeLong, 1971; Evoy & Cohen, 1971). For studies of this kind the nervous system of invertebrates has two attractive features. Firstly, many invertebrates are well known for having single motoneurons and interneurons which may be recognized in different animals of the same species. Secondly, most invertebrates show numerous stereotyped activities.

In most studies of the neuronal control of stereotyped activities in invertebrates, there is an underlying assumption. This is that the neuronal mechanisms may serve as models which are applicable to the vertebrate and thus to the human nervous system. What is therefore important in any invertebrate study is to attempt to deduce the functions of the component movements of the stereotyped activity, so that the underlying neuronal mechanisms may be judged as applicable to vertebrate movements which fulfil a similar function. On this basis there is justification for studying a variety of stereotyped invertebrate activities and their neuronal control.

The most thoroughly studied invertebrate motor systems may be categorized as: 1) rhythmical activities such as cockroach walking, lobster swimmeret beating and molluscan feeding (Davis & Kennedy, 1972*a*,*b*,*c*; Pearson, 1972; Kater & Rowell, 1973); 2) escape behaviour such as the crayfish tail-flick or nudibranch swimming (Willows & Hoyle, 1969; Zucker, 1972); 3) postural control such as is seen in the crayfish abdomen (Evoy & Kennedy, 1967; Kennedy, Evoy, Dane & Hanawalt, 1967);

4) simple reflexes such as the crab eyestalk-withdrawal response (Sandeman, 1969). To this date there have been no studies on the neuronal mechanisms underlying a non-rhythmical, fast activity which is not an escape behaviour or a simple reflex.

A most conspicuous activity in most decapod crustaceans is antennular flicking. The antennules, however, also show postural modifications and a variety of withdrawal reflexes. In the hermit crab, preliminary observations showed that the antennules are flicked almost continuously, yet this activity cannot be regarded as rhythmical and there is no clear coordination between left and right antennules. Some features of flicking are fairly stereotyped from flick to flick although other features of flicking and other antennular activities are more variable. These observations suggested that a study of the factors influencing activity in antennular motoneurons might provide an interesting comparison with similar studies in other motor systems.

A large amount of behavioural, electrophysiological and ultrastructural evidence has accumulated to suggest that in many crustaceans the antennules are of primary importance to the chemoreceptive process (Bell, 1906; Cowles, 1908; Holmes & Homuth, 1910; Copeland, 1923; Hodgson, 1958; Maynard & Dingle, 1963; Laverack, 1964; Laverack & Ardill, 1965; van Weel & Christofferson, 1966; Ghiradella, Cronshaw & Case, 1968; Ghiradella, Case & Cronshaw, 1968*a*,*b*; Hazlett, 1968; Ache & Case, 1969; Hazlett, 1971*a*,*b*; Snow, 1973). The antennule chemoreceptors are considered to be distance chemoreceptors which possibly operate in conjunction with the maxillipeds to give an animal the capacity for directional chemosensitivity (Brock, 1926; Hazlett, 1968).

The receptors responsible for chemoreception are generally considered to be the aesthetasc setae which occur in closely spaced rows on the outer antennular flagellum (Laverack, 1964; van Weel & Christofferson, 1966; Ache & Case, 1969). In addition to chemoreceptors the antennule has mechanoreceptive sensilla, osmotically sensitive receptors and internal joint receptors (Krijgsman & Krijgsman, 1954; Hodgson, 1958; Sandeman, 1963; Laverack, 1964; Wyse & Maynard, 1965; van Weel & Christofferson, 1966). The basal segment also contains the statocyst.

Despite the mass of work on the sensory aspects of the antennule there exists only one detailed description of the antennular activities in a crustacean (Maynard & Dingle, 1963). This description is based solely on the lobster, *Panulirus argus*, and is directed towards describing and classifying these activities rather than determining their function.

In the lobster four types of antennular activities may be recognized: flicking, pointing, wiping and withdrawal (Maynard & Dingle, 1963). Although reports of antennular activities in other crustaceans are incomplete, these four types of activity can be easily recognized in a wide variety of decapods. Between different species, however, there is considerable variation in the form and frequency of antennular activities and in the morphology of the antennules. In these respects there is more similarity between hermit crabs and brachyurans than there is between brachyurans and macrurans (personal observation).

The present study gives: 1) a description of the surface structures of the antennular flagella with particular reference to possible sensory structures; 2) a detailed description of the antennular activities and of the component movements of antennular flicking; 3) an account of the physiology of the motor innervation and musculature controlling movements of the joints between the medial and distal segments and the distal segment and the outer flagellum; 4) a description of the patterns of activity in identified antennular motoneurons during the various forms of antennular withdrawal and during flicking; 5) an assessment of the central versus reflex control of antennular flicking.

Throughout this work attention has been focused on antennular flicking. It is suggested that antennular flicking is important to the chemoreceptive process because it could facilitate the exchange of water trapped around the densely-packed chemoreceptive, aesthetasc setae. A model is presented to account for the patterning of the motoneuronal activity underlying flicking. This model is unique in that it suggests that one of the component movements of a flick is dependent on sensory feedback from a preceding component movement. This and other aspects of the model are discussed in relation to the form and the proposed function of flicking.

II. MATERIALS AND METHODS

Specimens of *Pagurus alaskensis* (Benedict) were dredged off Waldron Island, San Juan Archipelago, Washington and maintained in running sea water at the Friday Harbor Laboratories. Most of this work was carried out at the Friday Harbor Laboratories but for high speed filming animals were flown to Edmonton, Alberta where they were maintained successfully in "Instant Ocean." Large (body length, 6 to 8 cm) crabs, both male and female, were used in these experiments.

(1) Surface Structures of the Antennular Flagella

The antennules consist of three segments, the most distal of which bears the inner and outer flagella. The distal portion of the distal segment, bearing the inner and outer flagella, was removed. In addition the inner flagellum was sometimes removed from the distal antennular segment. The antennular flagella were examined under a light microscope and those whose aesthetasc setae were densely surrounded with what appeared to be filamentous fungi were discarded.

Tissue was fixed at 12°C, either in phosphate-buffered 2.5% gluteraldehyde for 1 h, followed by phosphate-buffered 2% osmium for 1 h or directly in phosphate-buffered 2% osmium for 2 h. Following fixation the tissue was washed in distilled water, dehydrated through an ethanol series, taken through an ethanol - amyl acetate series to 100% amyl acetate and critical-point dried. Dried specimens were mounted on stubs and coated with gold and gold-palladium. Specimens were examined on a Cambridge Stereoscan S4 scanning electron microscope (SEM).

Under the SEM the shape and orientation of various setae were often distorted from that observed in fresh material. Thus SEM observations were always supplemented by examination of freshly-excised antennules with the light microscope.

In order to use the light microscope to count the pores in the exoskeleton, freshly-excised antennules were washed in several changes of distilled water (5 min) and then the flagella were dipped into a 0.1 M $AgNO_3$ solution for 60 sec. The antennules were then washed again in several changes of distilled water and dropped into Kodak Microdal-X developer. Staining was observed under a dissection microscope and, when satisfactory, the antennules were rinsed in 3% acetic acid solution and whole-mounted in sea water. This technique gave excellent staining of the 1 to 3 μm pores in the flagellar exoskeleton (Plate 18) as well as staining the sockets of most of the setae on the inner and outer flagella.

In order to count the number of aesthetasc setae, fresh outer flagella were boiled in 10% KOH for 5 to 10 min. The ventral exoskeleton was then dissected away, dehydrated, cleared in Xylene and whole-mounted in Permount.

(2) Analysis of the Antennular Activities

Close-up motion pictures were made of the antennular activities using a Milliken DBM_{54} 16 mm, cine camera. Flicking was filmed at 400 frames/sec while other activities were filmed at 200 frames/sec. In the presence of water currents the antennules are frequently flicked without additional stimulation. A variety of stimuli, all of which were applied to animals immersed in sea water, were used to elicit other antennular activities. These stimuli will be mentioned as the other activities are described.

For each crab the right antenna was removed at its base several days before filming. Just prior to filming an animal was removed from its shell and the mesial sides of its left eyestalk and antenna were blackened to contrast the outline of the antennules. During filming the crab was placed in a small Perspex tank of "Instant Ocean' which was continually aerated and maintained at 12°C. This tank was small enough to prevent a large crab from turning so that all films could be shot from the right-hand side of an animal.

High-speed films were analyzed using an L.W. Photo Optical Data Analyzer fitted with a frame counter. For all antennular activities measurements were made of the duration, temporal relationship and total angle change of the movements about the medial segment-distal segment and distal segment-outer flagellum joints. The outer flagellum undergoes considerable distortion during flicking and thus the border of its large basal segment was selected as a reference for measuring angular displacements. For a more critical examination of antennular movements drawings were made from single frames.

To simplify descriptions of antennular movements flexion is considered to be an angle change at a joint, which moves the more distal part of the limb in the direction towards which the aesthetasc setae point.

In order to establish whether flicking occurred in any easily recognizable temporal pattern, unmolested animals were filmed in circulating sea water (10°C) at 50 frames/sec, using a Bolex 16 mm ciné camera. The number of frames between flicks of antennules, both ipsilateral and contralateral, was measured and these numbers were converted to time (1 frame = 20 msec). Qualitative data on antennular activities were derived from repeated examination of many film sequences and from direct observations.

Of primary interest in all the data is the amount of variation within a set of measurements. Calculations of the standard deviation and coefficients of variation are thus based on the equation S.D. = $\sqrt{(S.S./N)}$ rather than the equation S.D. = $\sqrt{(S.S./N-I)}$ (Sokal & Rohlf, 1969).

(3) Motor Innervation and Musculature

(a) Saline

The antennular muscles were found to be extremely sensitive to osmotic pressure. The following saline was developed on the basis of the osmolarity of sea water samples taken from the holding tanks (av. 885 mOsm) and of fresh blood samples taken from six animals (av. 870 mOsm). In accordance with the ionic analyses of hermit-crab blood (Robertson, 1953), the saline was made 30 mM with respect to sulphate ions. The following number of millimoles of components were dissolved in one litre of distilled water in this order: 463 NaCl; 8 KCl; 10 $MgSO_4$; 20 $CaSO_4$; 10 Tris buffer. The pH was then adjusted to 7·4 with conc. HCl and NaOH. This saline averaged 887 mOsm and in it muscles frequently gave good responses for up to 4 h. The data reported are restricted to those collected within the first 1·5 h of any experiment.

(b) Dissection

Both left and right antennules were used. Antennules were excised at the base of their proximal segment and placed in a small Petri dish of saline. This was maintained between 12 and 14°C by circulating sea water around the Petri dish. The bottom of the dish was covered with Sylgard 184 Encapsulating Resin (Dow Corning) which allowed rigid pinning and transillumination of the preparation.

In life the antennules are rotated frequently but in narcotized animals the aesthetasc setae point ventrally. The side which bears the aesthetasc setae will thus be referred to as the ventral side of the antennule.

Using a sliver of razor blade the exoskeleton of the proximal segment was sliced longitudinally along the dorsal and ventral surfaces. The proximal segment was then pulled apart and the half containing the statocyst was removed by cutting its membranous attachment with the medial segment. The remaining half contains the nerves supplying the medial and distal antennular segments.

Cochran (1935) described and gave numbers to three muscles of the medial and distal segments of a brachyuran antennule: muscle 30 (musculus productor$_3$ I antennae); muscle 31 (musculus reductor$_3$ I antennae); muscle 32 (musculus reductor$_4$ I antennae). Dissection showed that in the antennule of the hermit crab three muscle groups of similar function to Cochran's muscles 30, 31 and 32 may be recognized, but two of these are subdivided into two separate muscles giving a total of five muscles in three muscle groups 30, 31 and 32. Muscle group 30 raises the distal segment while 31 depresses the distal segment and 32 depresses the outer flagellum.

Muscle groups 31 and 32 were exposed by longitudinally shaving the exoskeleton from the mesial side of the medial and distal segments, respectively. Muscle group 30 was exposed by similarly removing the exoskeleton from the lateral side of the medial segment. Such cuts were made from the arthrodial membrane at the distal end of a segment to about one-third to one-half the length of the segment.

Each muscle group is connected to the basal exoskeleton of the more distal limb by a single tendon (Fig. 1). A small wedge of this exoskeleton attached to the tendon of a muscle group was cut and the rest of the more distal limb was excised. The antennule was stapled to the Sylgard with insect pins so that the muscle group was exposed dorsally and the wedge of exoskeleton was attached to a tension transducer. The remaining half of the proximal segment was pinned inner side dorsally and the muscles overlying the nerves in the proximal segment were removed by cutting their distal and proximal attachments and carefully severing associated nerve branches.

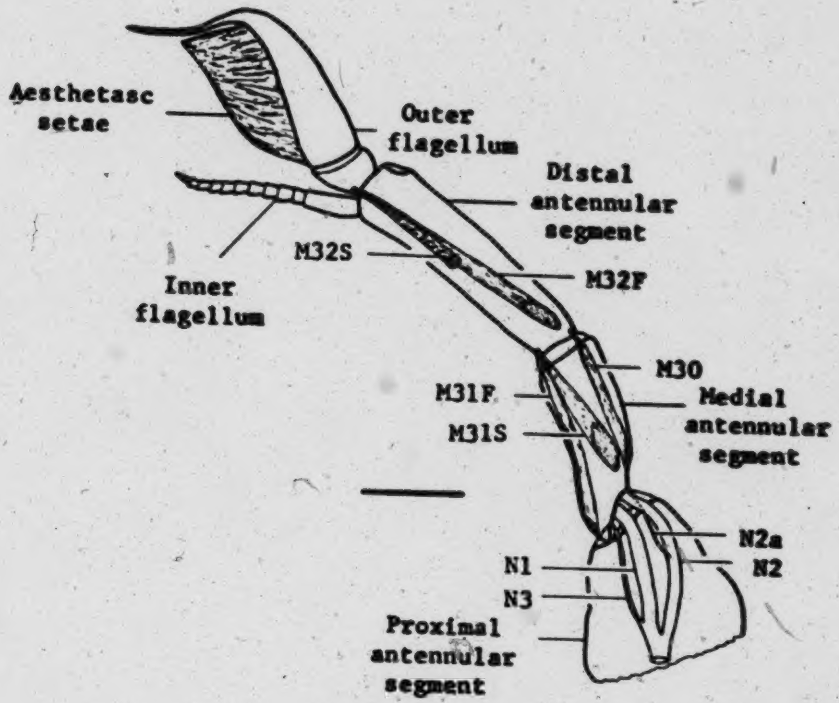
(c) Stimulation and recording

Initially the nerves present were stimulated with fine silver hooks while recording intracellularly in muscle groups 30, 31 and 32. It soon became apparent that the proximal portion of one nerve contained all the motor axons, and a fine suction electrode was used in most succeeding preparations. Motor axons were differentiated by exploiting their differences in threshold to electrical stimulation. Frequently intracellular junction potentials were simultaneously recorded in two fibres of one muscle group using an Adak Electronics FET follower and a WPI M4a electrometer. Whole-muscle tension was monitored using an RCA

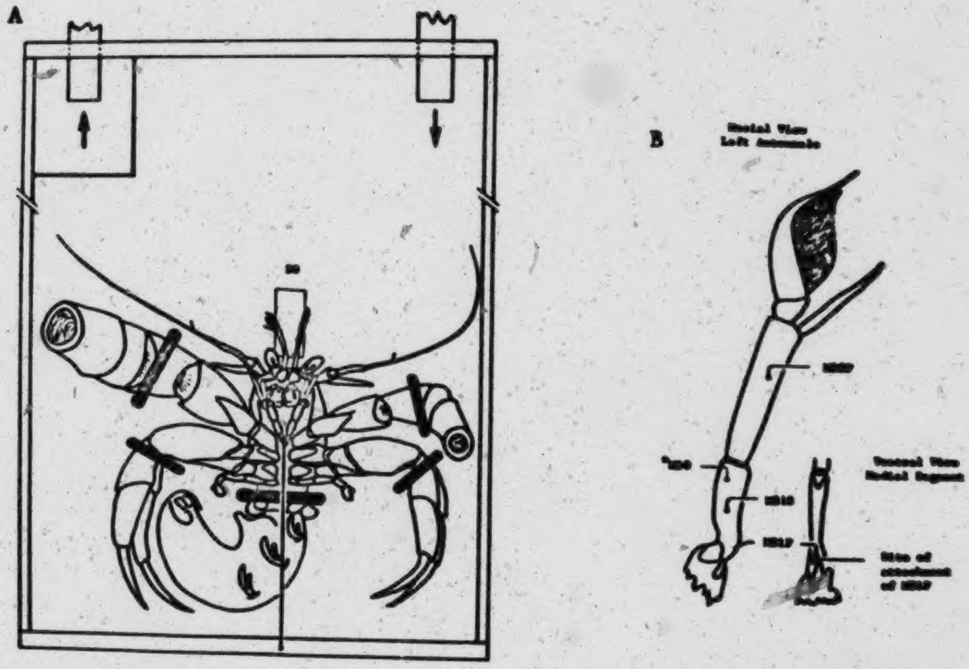
Fig. 1. Mesial view of the right antennule. Showing the muscles of the distal and medial segments. The mesial half of the proximal segment exoskeleton (see text) has been rotated 180° to expose the antennular nerves. Scale — 2 mm.

Fig. 2. Method of recording electromyograms from the antennules of intact animals. A: crab stapled in the experimental chamber. Arrows indicate the inlet and outlet sea water supply. Note the Sylgard block (SB) beneath the antennules. Myogram electrodes have been omitted for clarity. B: mesial view of the left antennule and ventral view of the medial segment, showing the sites of placement of the myogram electrodes. The myogram electrode for muscle 30 (see: *M30) is introduced through the exoskeleton of the lateral side of the medial segment.

(1)


Aesthetasc setae
Outer flagellum
Distal antennular segment
M32S
Inner flagellum
M32F
M30
M31F
Medial antennular segment
M31S
N2a
N1
N2
N3
Proximal antennular segment

(2)


A
B
Medial View
Left Antennule
Ventral View
Medial Segment
Site of attachment

5734 tension transducer. Recordings were displayed on a Tektronix 565 oscilloscope and photographed directly using a Nihon Kohden PC-2A continuous recording camera.

(d) Histology

The microanatomy of the antennules was determined by dissection of fresh material, thin sectioning of Epon-embedded nerves and serial sectioning of wax-embedded antennules. For measurements of axon diameters the nerves of the basal segment were fixed in a 2·5% phosphate-buffered gluteraldehyde and 1% phosphate-buffered osmium. These were embedded in Epon and 1 μm sections were stained in Richardson's stain. For serial sectioning the entire contents were withdrawn from the old exoskeleton of pre-moult antennules. These were secured to strips of Sylgard and fixed in Bouin's for 24 h. They were then dehydrated, cleared in toluene and embedded in wax. Serial sections were taken at 15 μm and stained in Masson's trichrome (Pantin, 1948).

(e) Sarcomere measurements

Sarcomere lengths were measured by excising the antennules of narcotized animals at the base of the proximal segment. All three muscle groups were then exposed as described above and the antennules were secured to Sylgard strips in the position which they adopt in narcotized animals. Antennules were fixed in 2·5% phosphate-buffered gluteraldehyde for 1 h and washed in sea water. The muscles were dissected out onto glass slides, teased apart and the mean sarcomere lengths were calculated in each muscle from direct measurements using the light microscope.

4. Patterns of Activity in the Antennular Motoneurons

It proved difficult to consistently record activity from the antennular motoneurons in whole animals. Electromyograms from the antennular muscles were thus used to monitor motoneuronal activity during the performance of various antennular activities.

(a) Preparation of animals for recording

The method used for recording from the antennules of whole animals is shown in Figure 2. Crabs were removed from their shells and held ventral side up in a 15 X 9.5 X 4 cm plexiglass chamber which was continuously supplied with running sea water (10 to 12°C). Large staples, made from bent nails, were used to secure crabs to the wax bottom of this chamber. Staples were placed across the joint between the meropodite and the carpopodite of the left and right pairs of walking legs, the carpopodite of the left and right chelipeds and the posterior portion of the thorax in the region of the 5th pereiopods. The chelipeds were also prevented from opening by gloves made from rubber tubing (Fig. 2A).

To enable the antennules to be viewed from above the preparation, the distal segments of the endopodites of the 3rd maxillipeds were tied together with thread and both appendages were drawn towards the abdomen and secured in this extended position. The antennae were also secured in an extended position by pinning the antennal flagella to the bottom of the chamber.

In this condition the base of the antennules is about 2 to 3 mm above the bottom of the chamber. To facilitate implantation of the electrodes, a block of Sylgard, 4 X 7 X 20 mm, was pinned underneath the antennules (Fig. 2A). This was easier to do if the eyestalks were first spread and prevented from returning to their anterior orientation by insect pins. The antennules were stapled to the block of Sylgard with insect pins and the electrodes implanted. The antennules and eyestalks could then be freed and the block of Sylgard removed.

Animals suffered no apparent ill effect even after being maintained in a recording situation for up to 72 h. In the presence of water currents the antennules were flicked frequently and showed the various types of antennular withdrawal. The frequency of flicking could be increased by pipetting a little distilled water into the inlet of the experimental chamber or by initiating additional water currents in the chamber by alternately squeezing and releasing the inlet hose. When the endopodites of the 3rd maxillipeds were released many animals showed antennular wiping.

(b) Recording technique

The motor innervation and anatomy of the antennular muscles of the medial and distal antennular segments are described in Section (3), Chapter III. The patterns of motor innervation of the antennular muscles and the facilitatory properties of their neuromuscular synapses enables the identification of each motoneuron from electromyogram recordings within any muscle group. Each myogram electrode consisted of two 15 to 20 cm lengths of 50 μm diameter insulated copper wire. These lengths were twisted around one another and painted with Insul-X and then xylene. Each wire was connected to one differential input of a Tektronix 122 preamplifier. Prior to each recording the distal end of each electrode was cut squarely to ensure that only the tip of the component wires were exposed. Recordings were displayed on an oscilloscope and photographed directly or taped. During flicking, activity is first seen in muscle 31F. This activity was used to trigger sweeps of the oscilloscope to provide fast-sweep displays of activity in all muscles during a flick (e.g. Fig. 20).

Up to four electrodes were sometimes used to simultaneously monitor electrical activity in muscles 30, 31F, 31S and muscle group 32. The optimum placement of an electrode for each of these sources is shown in Figure 2B. Using an insect pin a small hole was poked in the semi-transparent exoskeleton of the antennule and the tip of an electrode was inserted into the muscle under visual control. The exoskeleton of the distal segment is relatively opaque but this segment is thin and contains only muscle group 32 which is innervated by two motoneurons that are easily distinguishable in myogram recordings. With practice the hole size could be judged so that the Insul-X coating on the electrode plugged most of the hole and coagulating blood rapidly plugged the remainder. On the basis of visual inspection the electrodes seldom appeared to impede any of the antennular activities.

Most experiments lasted only a few hours but in some cases recording was continued for up to 72 h requiring only the occasional adjustment of electrode position to restore a tolerable signal/noise ratio. For short periods of time it was possible to record from freely moving animals but these animals usually dislodged the electrodes when they wiped their antennules.

Although electrodes were rarely dislodged from the antennules, small displacements of their tips often resulted in changes in the waveform of the record. When this occurred the high-frequency activity in fast muscles 31F and 32F was sometimes recorded as a complex waveform in which individual excitatory junction potentials (EJPs) could not be clearly distinguished. Such records were typical when a poor signal/noise ratio was being obtained. Adjustment of the electrode position usually restored the clarity of individual EJPs as well as improving the signal/noise ratio.

(5) Central Patterning and Reflex Control of Antennular Flicking

Electromyograms were recorded from the antennules of partially restrained animals as described above. The effects of a variety of manipulations of the antennules and operations on the antennules, antennae, eyestalks and circumoesophageal connectives, on antennular flicking, were tested. These manipulations and operations will be described where applicable in the text.

In the course of these experiments it was necessary to directly stimulate flexor muscle 31F. A second myogram electrode was implanted in muscle 31F about halfway along the ventral surface of the medial segment. This electrode was identical to the recording electrodes described above, except that it consisted of two 100 µm diameter insulated copper wires.

It was also necessary to record from some of the antennular nerves in the proximal segment of excised antennules. These nerves were exposed as described above (Section 3 *(b)*, Chapter II).

Recordings were made by raising whole nerves on monopolar or bipolar platinum hook electrodes into a mixture of paraffin oil and Vaseline.

III. RESULTS

(1) Surface Structures of the Antennular Flagella

(a) Morphology of the antennular flagella

The antennules arise below and slightly mesial to the eyestalks. They consist of three segments, the most distal bearing the inner and outer flagella. Neither the outer nor the inner flagellum contains muscles, and only the outer flagellum (OF) can be moved independently of the distal antennular segment (DS) (see Fig. 1).

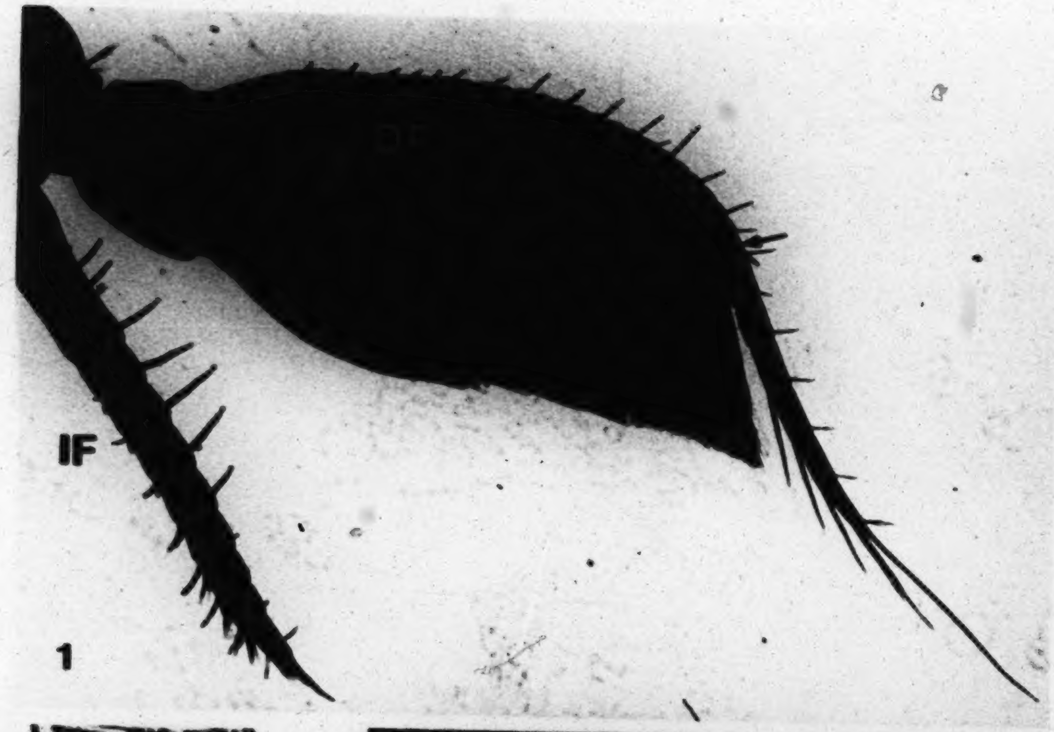
The OF resembles a cone which has been bent so that its axis of rotation is approximately sickle-shaped (Plate 1). It has a relatively large basal segment (approx. 500 μm long) which does not bear any setae. Distal to the basal segment are 34 to 40 short (70 to 80 μm) segments of which the most proximal 8 to 13 are partially fused. The most distal extent of fusion is along the dorsal surface of the short segments and the extent of lateral and mesial fusion between segments increases proximally (Plates 2 and 4). The many unfused short segments give the OF considerable mechanical flexibility. The distal one-quarter of the OF is composed of 6 or occasionally 7 long, thin segments (150 to 250 μm long). In comparison with the short segmented region, the OF is relatively inflexible across these long thin segments. What is considered to be the most distal short segment is intermediate in length (approx. 120 μm) between the long, thin segments and the other short segments (Plate 1).

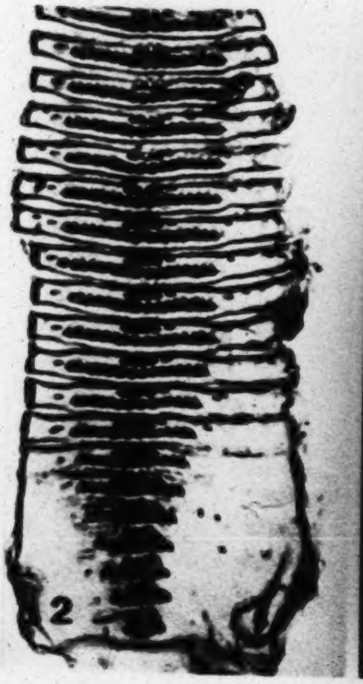
The inner flagellum (IF) is a pencil-shaped structure with a long (700 to 1000 μm) basal segment and 8 to 10 shorter (150 to 280 μm)

Plate 1. Mesial view of the outer (OF) and inner (IF) flagella of the left antennule. Arrow indicates what is considered to be the most distal short segment. X 37.

Plate 2. Preparation of the ventral exoskeleton of the outer flagellum, showing the long and short rows of aesthetasc setae on each short segment. Note the fusion between the most proximal 7 segments. X 60.

Plate 3. Ventral view of an outer flagellum and the distal portion of the distal antennular segment showing the medial part in the aesthetasc setae. The inner flagellum has been removed. X 27.


OF
IF
1


2

segments (Plate 1). The IF is borne at an angle of 20 to 30° to the axis of the DS, such that it is closest to the most concave aspect (ventral side) of the OF.

(b) Surface structures of the outer flagellum

1. Aesthetasc setae

One long and one short row of partially recumbent aesthetasc setae are borne on each of the short segments of the OF (Plate 2). In addition, about 4 aesthetascs occur on the proximal end of the most proximal long, thin, distal segment. On any short segment the short row occurs distal to the long row, the inter-row distance being 20 to 25 µm. There are usually 3 to 4 aesthetascs/short row. In contrast, the number/long row varies from 4 on the most proximal and most distal short segments up to a maximum of 13 to 18 on short segments 12 to 25 (Plate 2). There are thus 400 to 600 aesthetascs/OF. Within any row the distance between the setae bases is often less than 10 µm but a medial part divides the distal portions of the aesthetascs into a mesial and a lateral group (Plate 3). Because of the curvature of the OF, the distal portions of the aesthetascs of adjacent rows are bunched together.

Each aesthetasc is borne in a single socket within which the setal diameter is considerably reduced (Plate 5). Where an aesthetasc emerges from the socket there is a distinct swelling or ampulla. This swelling is less pronounced on the proximal side of each seta where the swelling begins almost within the socket (Plate 5).

The aesthetascs measure from 700 to 1500 µm in length and are about 18 to 25 µm in diameter. The diameter within any animal is quite

Plate 4. Dorsolateral view of the outer flagellum showing the most distal extent of fusion between the short segments. Note the absence of dorsal setae on the fused short segments. X 120.

Plate 5. Base and socket of an aesthetasc seta. Note the swelling at the base of the seta (arrow). X 2180.

Plate 6. The third and fourth most proximal annulations on the wall of an aesthetasc seta. X 1540.

Plate 7. Typical annulations about halfway along an aesthetasc seta. X 1400.

Plate 8a. Ventral view of the aesthetasc seta showing the many periodic annulations along the setal walls. Arrows mark places where bending of a seta appears to occur at an annulation. X 590.

Plate 8b. Tip of an aesthetasc seta showing the absence of any pore-like structure. X 8960.

Plate 9. Tip of an accessory seta. Note the distinct terminal pore. X 25200.

constant. For the first 50 to 150 μm of their length they have a slightly smaller but irregular diameter (Plate 10). For the distal one-third of their length they taper slowly to their tips which are approximately 2 μm in diameter and do not appear to be penetrated by a pore (Plate 8b).

At about 100 to 250 μm from their bases ill-defined annulations can be seen on the walls (Plate 6). More distally these give way to clearly defined annulations (Plate 7) which give each seta a segmented appearance (Plate 8a). These annulations occur with a regular periodicity of 45 to 55 μm and the most distal annulation is found at this distance from the tip (Plate 8a). It is interesting to note in Plate 8a, that sometimes bending of the aesthetascs seems to occur maximally at the annulations. It is, however, possible that this effect is a fixation or dehydration artifact.

2. *Accessory setae*

The accessory setae are found only on the lateral side of the OF. There are usually 8 to 14 accessory setae on each OF, one seta usually occurring about every third or fourth short segment (Plates 10 and 15). Accessory setae are found only on the most distal fused, short segments. Each accessory seta is borne in a socket which lies on the distal extremity of the short segment, about 30 to 50 μm from the base of the most lateral aesthetasc of the row (Plate 10).

In fresh material the accessory setae are only slightly recumbent. They measure 40 to 80 μm in length and about 3 to 5 μm in diameter and have a single annulation about one-third of the distance from their base. In both the SEM and the light microscope their shape is somewhat

Plate 10. Lateral view of some short segments of an outer flagellum. Note the accessory setae near the end of some aesthetasc rows (black arrows) and the exoskeletal pores (white arrows). X 220.

Plate 11. Exoskeletal pit on the surface of an outer flagellum. X 25700.

Plate 12a. Rows of exoskeletal pores on the mesial side of an outer flagellum. Note the spatial relationship to the aesthetasc rows (arrow). X540.

Plate 12b. Material within a single exoskeletal pore. X8200.

Plate 12c. Light micrograph of a single exoskeletal pore showing the spherical cavity below the exoskeleton surface (arrow). Note the canal which appears to connect this cavity with the inner surface of the exoskeleton. X 1170.

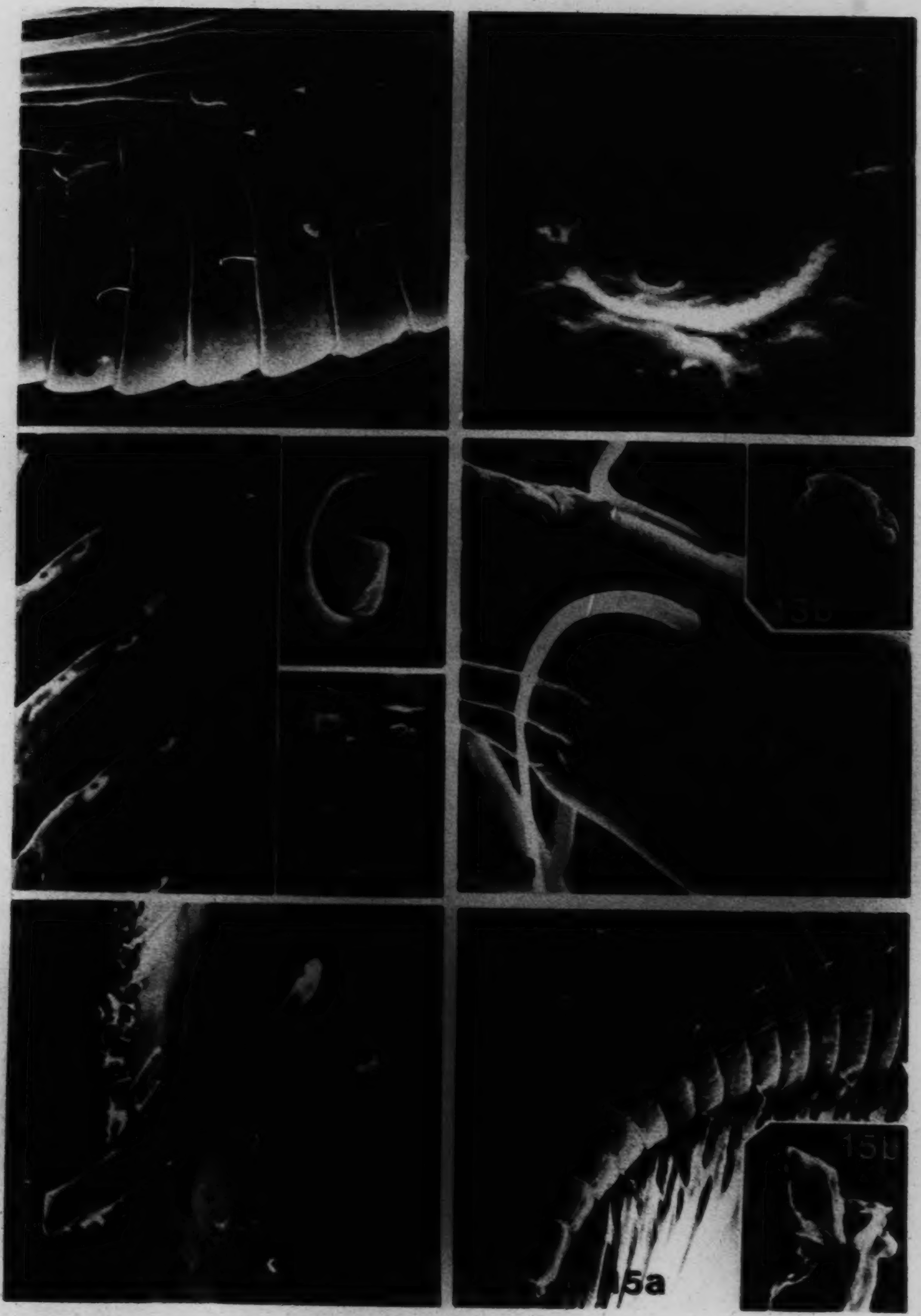
Plate 13a. Exoskeletal pores on the mesial side of the most distal segment of an outer flagellum. X 1100.

Plate 13b. Bulbous process extending from one of the exoskeletal pores shown in Plate 13a. Note the infolding of the surface of the process. X 9130.

Plate 14. Dorsal view of a proximal dorsal seta. Note the single annulation (arrow) and the irregularly shaped tip. X 1200.

Plate 15a. Dorsolateral view of an outer flagellum. Note the accessory setae, the single line of lateral-mesial setae (black arrows) along the lateral side of the flagellum and the dorsal setae (white arrows). X 109.

Plate 15b. Tip of a dorsal seta on a distal segment of the outer flagellum. X 8200.


13b
5a
15b

irregular but they uniformly show only slight taper to within 2 to 3 µm of their tip. At their tips they have a distinct terminal pore with an internal diameter of about 0·5 µm (Plate 9).

3. *Exoskeletal pits and pores*

(i) *Pits*. These structures are well distributed over the exoskeleton of the OF, IF and DS. They appear as a small hole of 0·1 to 0·2 µm in diameter which occurs in the centre of a dome-shaped depression of about 1·0 µm in diameter (Plate 11). Focussing through the antennular exoskeleton with the light microscope reveals many lines which follow an irregular course and appear to traverse the exoskeleton. It seems likely that these are tiny ducts and that the pits are the openings of these ducts to the surface.

(ii) *Pores*. These structures have been seen on the IF and OF but not on the DS. On the OF they occur on both sides of the short segments and on the mesial side of the long, thin, distal segments (Plates 10, 12a and 13a) but not on the 5 most proximal, fused, short segments.

On the short segments pores occur singly or in rows of 2 and rarely 3, 10 to 20 µm from the distal edge of each segment and 30 to 80 µm from the end of the long rows of aesthetascs. Within a row, the pores are separated by 20 to 40 µm (Plate 12a). There are usually 30 to 45 pores on each side of the short segments of this region.

On the long, thin, distal segments there are 5 to 12 pores each of which occurs in a slight depression of the exoskeleton surface (Plate 13a). Usually 3 to 4 of these occur on the mesial side of the most distal segment (Plate 13a) while none have been seen on the most proximal long, thin, distal segment. On the other long, thin, distal segments the pores occur singly or in groups of 2 or 3 on the ventromesial surface, 20 to 40 µm from the distal edge of each segment.

Each pore is 1 to 3 µm in diameter and always contains a structure which because of its irregular shape is considered as either a secretion or a cellular process (Plate 12b). In some specimens a more regularly-shaped bulbous process could be seen extending from each pore of the long, thin, distal segment. The surface of this process seemed to be infolded in several places suggesting that it may be cellular (Plate 13b). Light microscope observations suggest that just below the exoskeleton surface each pore opens into a spherical cavity of 9 to 10 µm in diameter and that a canal connects this cavity with the inner surface of the exoskeleton (Plate 13c).

4. *Dorsal setae*

A single line of setae occurs along the dorsal (convex) surface of the OF (Plate 1). Usually only the most distal of the fused, short segments and all but 4 to 6 of the unfused, short segments bear a single dorsal seta (Plate 4). There are thus usually 20 to 26 dorsal setae/OF.

Each dorsal seta arises from a socket near the distal extremity of its segment of origin (Plates 1, 14 and 17a). The setae on the proximal short segments are shorter, more recumbent and are different in shape from the setae on the more distal short segments and the long, thin, distal segments (Plate 1). Along the OF, however, there is a gradual morphological gradation between the two types which defies attempts to be more specific regarding their precise location.

In fresh material the more proximal setae are orientated at an angle of 20 to 45° to the axis of their segment of origin but during preparation for the SEM they become more recumbent and appear to make contact with the surface of the more distal segment (cf. Plate 1 with 14). The most proximal are 40 to 50 μm in length and 7 to 9 μm in diameter at their base. About 20 to 30 μm from their base they have an annular bulge from which they taper to an irregularly-shaped tip (Plate 14).

In fresh material the setae of the distal, short segments and the long, thin, distal segments are relatively straight and are orientated at an angle of 80 to 90° to the axis of their segment of origin (Plates 1 and 17a). Preparation for the SEM usually results in these setae becoming curved and adopting a more recumbent orientation (Plate 15a). They are 4·5 to 7 μm in basal diameter and 110 to 270 μm in length, the longest usually occurring on the more distal short segments (Plate 1). About halfway along their length these setae have a single annulation from which they taper to a bevelled tip. In the SEM they often appear to have material extending from a 0·5 to 1·0 μm pore in the bevel surface (Plate 15b). This material cannot be seen in the light microscope and is considered to be hardened exudate which has arisen during preparation of the tissue for the SEM.

5. *Lateral-mesial setae*

A single line of setae occurs along the lateral and mesial sides of the OF (Plates 10, 15a and 18). These lines do not extend over the 6 most proximal short segments which are usually fused along either side. Each line is made up of two types of setae; type I occurring only on the short segments and type II occurring only on the long, thin, distal

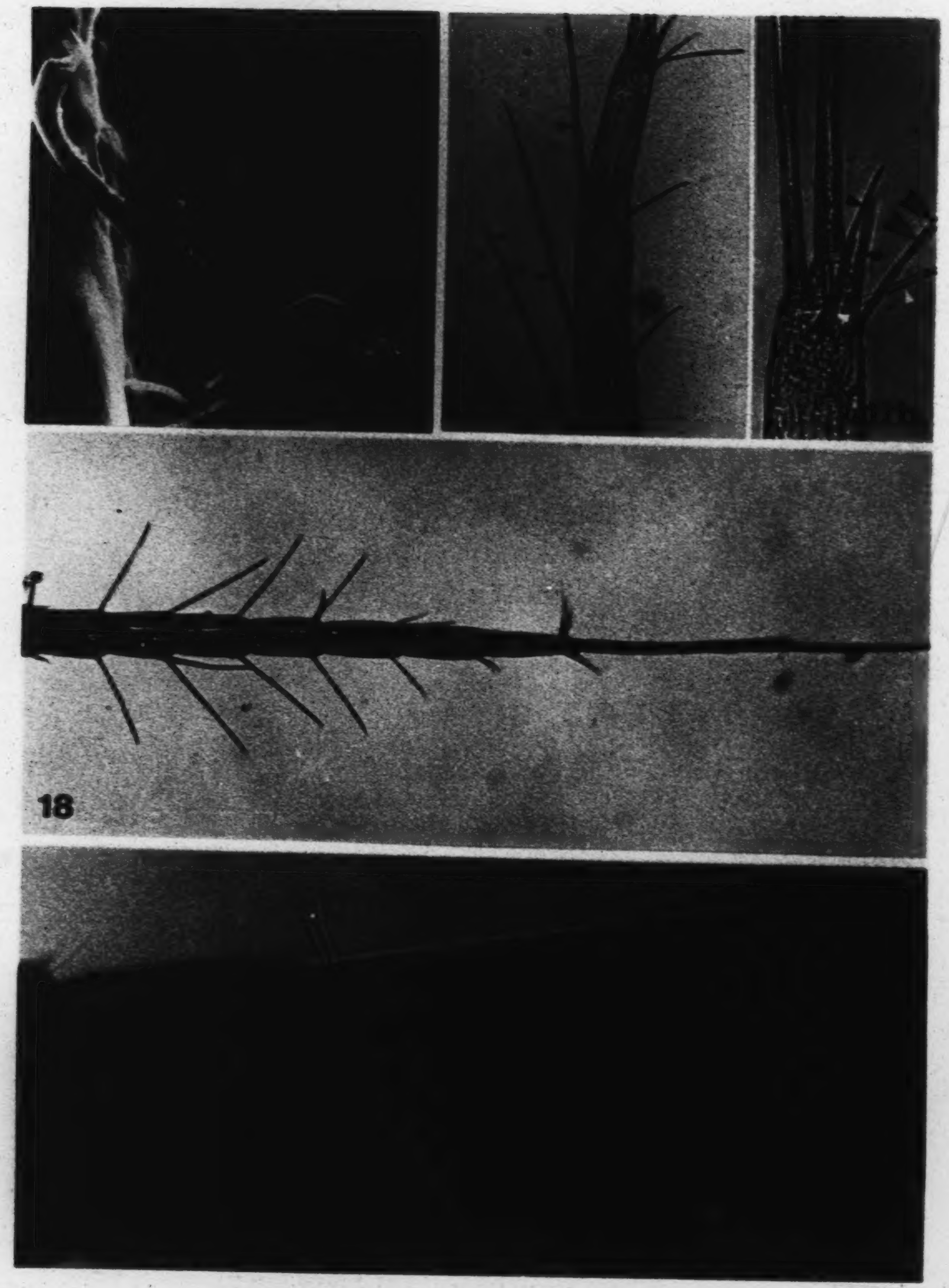
Plate 16. Lateral view of a type I lateral-mesial seta. Note the indistinct annulation and irregularly-shaped tip. X 2040.

Plate 17a. Lateral view of some of the long, thin, distal segments of an outer flagellum showing ventral (arrows) and dorsal setae. X 102.

Plate 17b. Higher magnification of the most distal segment of an outer flagellum. The lateral-mesial setae (small black arrows), dorsal seta (large black arrows) are distinguishable from two other small setae (white arrows) which occur on the dorsal and lateral surfaces, respectively. The bases of the larger setae can be seen on the tip of the segment. X 260.

Plate 18. Ventral view of the long, thin, distal segments of an outer flagellum showing the type II lateral-mesial setae. X 48.

Plate 19. The distribution of exoskeletal pores (dark dots) along the mesial surface of the fourth, fifth and sixth segments of the right inner flagellum. Pores have been stained with the silver nitrate - Microdal-X technique. X 200.



18

segments.

(i) Type I. There are usually 17 to 23 type I setae on the mesial side of the OF and 16 to 19 on the lateral side. Each seta arises from a socket near the distal extremity of its segment of origin (Plate 15a). Under the SEM these setae are often seen in contact with the exoskeleton of the more distal segment (Plate 16). In contrast, in fresh material they are orientated at 25 to 45° to the axis of their segment of origin. Furthermore, they do not make contact with the more distal segment even when a lateral or mesial bend of 90° is imposed across the short segmented portion of the OF.

The type I setae are 40 to 65 µm long and about 5 µm in basal diameter. They have a single annulation, 10 to 15 µm from their base. From this they taper to a point about two-thirds of their length from their base and then become wider, curving away from the exoskeleton surface to end in an irregularly-shaped tip (Plate 16).

(ii) Type II. On each side of the OF one seta arises from a socket near the distal extremity of each long, thin, distal segment (Plate 18). Usually either the second, third or fourth most distal segment bears 2 setae either on the mesial or lateral side. Thus on an outer flagellum with 6 long, thin, distal segments there are 13 type II setae. These setae are less recumbent than the type I setae. In life, they are orientated at 30 to 70° to the axis of their segment of origin (Plate 18).

The length of the type II setae increases gradually from about 60 µm on the most distal long, thin, distal segments to 300 to 400 µm on the most proximal. Concurrently their basal diameter changes from 4·5 µm on the most distal to 10 µm on the most proximal segment. The shape and tip morphology of these setae resembles that described for the dorsal setae of the distal segments of the OF.

6. *Ventral setae*

These setae are found only on the ventral side of the long, thin, distal segments of the OF (Plate 1). All but the most proximal long, thin segment bear one and rarely two ventral setae each of which arises from a socket near the distal extremity of each segment. There are thus usually 5 ventral setae/OF.

In fresh material the ventral setae are borne at an angle of 10 to 20° to the axis of their segments of origin (Plate 17a). They are 9 to 14 µm in basal diameter and 350 to 600 µm in length but within any animal there is no regular gradation in these parameters along the long, thin segments. The shape and tip morphology of these setae resembles that described for the dorsal setae of the more distal segments of the OF.

7. *Setae of the distal segment*

The most distal segment of the OF bears 3 to 5 large setae on the tip and more proximally 4 to 5 smaller setae (Plate 17b). The shape and tip morphology of these setae resembles that described for the dorsal setae of the more distal segments of the OF.

Amongst the smaller setae one can define what appears to be the lateral-mesial setae and the dorsal seta of the distal segment. In addition, there is a small seta which arises near the dorsal seta and sometimes a second small seta which arises near the lateral or the mesial seta (Plate 17b). The small setae are 3 to 5 µm in basal diameter and 60 to 150 µm in length.

The longest large seta occurs on the dorsal portion of the tip and is usually 15 to 20 μm in basal diameter and 1100 to 1500 μm in length (Plate 1). The large setae become shorter as one moves ventrally across the tip and the shortest seta is considered to be the ventral seta of the distal segment (Plate 1).

(c) Surface structures of the inner flagellum

1. Exoskeletal pits and pores

As mentioned above the IF has a number of pits and pores in its exoskeleton. These are morphologically similar to those described on the OF. The pits are well-distributed over the surface of the IF but the pores are found only on the mesial side of each segment (Plate 19). In all there are 20 to 35 pores occurring on each IF (Table 1). On the most proximal segment the pores are scattered along the distal one-half of the segment. On subsequent segments they are usually within 50 to 100 μm of the distal extremity of each segment and are often found in groups near the bases of the setae of the mesial row (Plate 19).

2. Setae

There are two longitudinal rows of setae on the IF: a lateral and a mesial row. On the proximal segment the lateral row actually lies along the dorsolateral surface. Across subsequent segments it spirals around the IF so that it ends on the dorsal surface of the distal segment (Plate 21). Conversely, the mesial row spirals around the IF until on the distal segments it occupies the ventral surface of the IF (Plate 20).

The setae of these rows may be divided into two types primarily on

Plate 20. Mesial view of the right inner flagellum. Note the spiralling of the longitudinal row of setae around the inner flagellum and the larger type II and smaller type I setae on the more proximal segments. This specimen is unusual in not having a long, recumbent type II seta on segments 2 and 4. X 91.

Plate 21. Lateral view of the right inner flagellum. Note the larger type II and the smaller type I setae on the more proximal segments and the reduced size of the type II setae on the distal segments (arrows). X 78.

Plate 22a. Pore in the tip of a type I seta of the inner flagellum. X 17500.

Plate 22b. Pore and "tongue-like" protrusion on the tip of a type II seta of the inner flagellum. X 17500.

Plate 23. Ventral view of the first to seventh segments of the right inner flagellum. Black arrows indicate the small groups of type I setae which are ventrally displaced from the mesial row. Note the long, recumbent type II setae of the mesial row which occur on segments 2 and 3 (white arrows) and the erect posture of the type I setae of the lateral row. X 88.

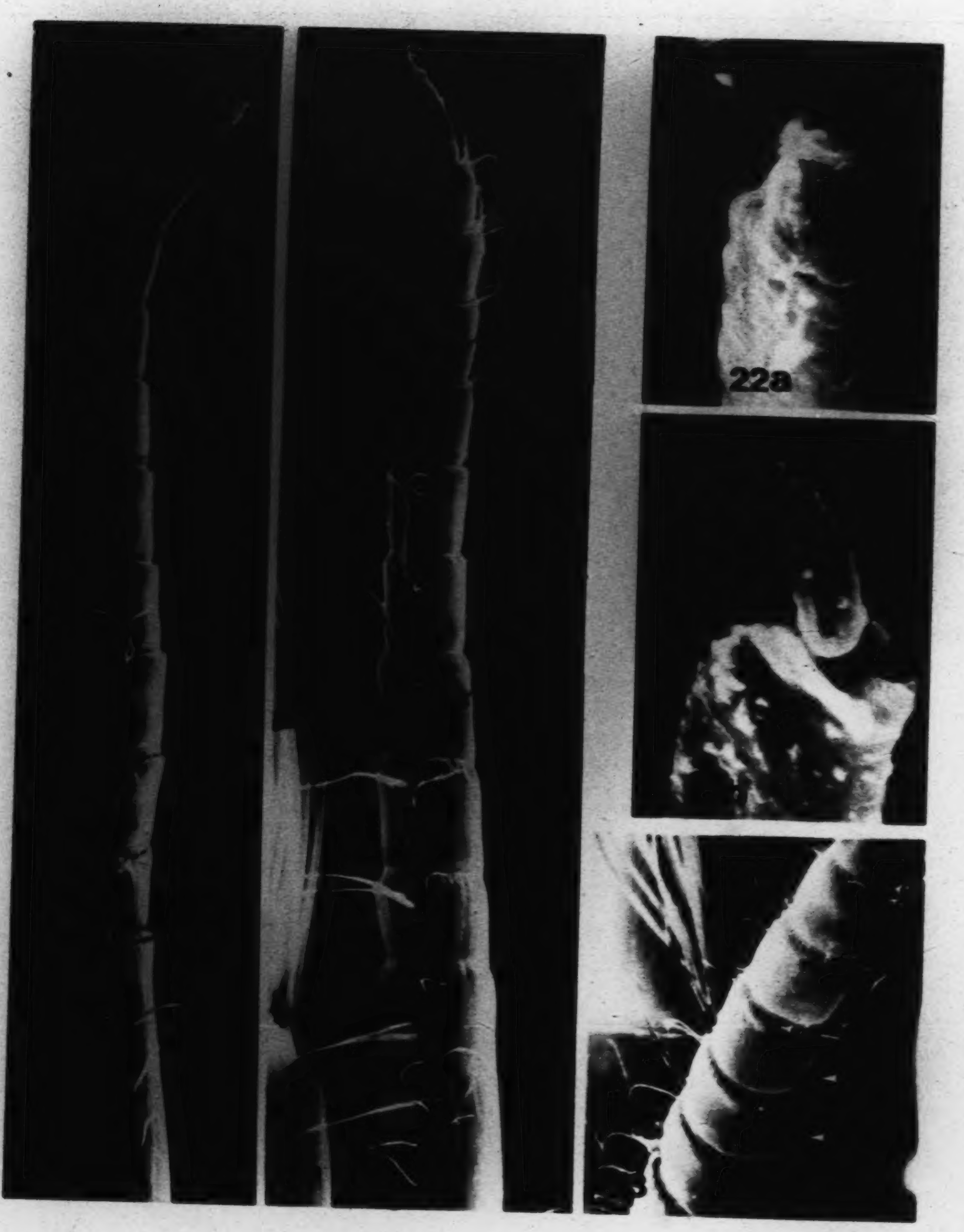

22a

Table 1. *Distribution of setae and exoskeletal pores on the inner flagella*

	Setae of the mesial row						Setae of the lateral row						Exoskeletal pores		
Specimen:	(1)		(2)		(3)		(4)		(5)		(6)		(7)	(8)	(9)
Setal Types: Segments	I	II	I	II	I	II	I	II	I	II	I	II			
1	10,(2)	4	11,(1)	4	14,(2)	4	8	4	10	6	11	2	5	1	7
2	3,(2)	2	3,(3)	1	2,(1)	3	1	1	2	1	1	2	1	2	4
3	3,(1)	2	3,(1)	2	2,(3)	2	2	1	2	1	3	1	3	2	2
4	3,(2)	2	3,(2)	2	3,(1)	2	1	1	1	1	1	1	4	2	3
5	2,(2)	4	3,(2)	3	3,(2)	1	1	1	1	1	1	1	3	2	3
6	3,(2)	3	4,(1)	3	3,(2)	2	1	2	1	1	1	1	3	3	3
7	5	4	4	5	2,(2)	2	1	1	1	1	1	1	4	2	3
8	5	7	4	5	3	3	2	1	2	1	1	1	2	3	3
9	5	7	5	5	3	4	1	1	1	1	1	1	2	1	3
10	15		14		5	5	1	1	1	1	1	1	3	4	3
11					13		1	1			1	1			
Total	55*	35*	50*	30*	53*	28*	20	15	22	15	23	13	30	22	34

Numbers in parentheses are additional type I setae which are ventrally displaced from the mesial row. On the distal segment only the total numbers of setae in the mesial row are given.

*Indicates the total numbers of type I and type II setae in the mesial row, not including those setae on the distal segment.

the basis of tip morphology. Type I setae resemble the accessory setae of the OF in that they have a distinct terminal pore with an internal diameter of 0·4 μm (Plate 22a). They are 50 to 250 μm in length and 4 to 9 μm in basal diameter. In contrast, the type II setae have a 1·0 μm pore which often has material extending from it (Plate 22b). This material is not visible under the light microscope and is considered to be exudate from the setae. Type II setae range from 3 to 20 μm in basal diameter and 50 to 1100 μm in length. In fresh material some type I and type II setae are erect while others are quite recumbent. Annulations have not been seen along either type I or type II setae.

(i) *The arrangement of setae in the mesial row.* The mesial row begins about one-third of the way along the proximal segment. On this segment there is a longitudinal row of 3 to 5 erect type II setae which are spaced about 100 μm apart. Ventral to this row there is a group of 12 to 16 type I setae (Plate 20). In fresh material 3 or 4 of this group are erect while others are recumbent. Generally a small row of 2 to 4 recumbent setae occur on the distal extremity of the proximal segments (Plate 20).

On the second segment there are 3 to 6 type I setae and 1 to 3 type II setae. The number of type I setae/segment on the more distal segments varies only slightly but the number of type II setae increases to 5 to 7 on the second most distal segment (Table 1).

Both type I and type II setae occur in a transverse row 10 to 30 μm from the distal extremity of each segment. With the exception of the distal segment, type II setae always lie dorsal to the type I setae (Plate 20). On segments 1 to 6 a small row of 1, 2 and sometimes 3 recumbent type I setae occur ventral to the row of more erect type I setae (Table 1 and Plate 23). The latter group of type I setae become more recumbent distally and this, combined with the spiralling of the mesial row ventrally around the IF, makes these two groups of setae indistinguishable on the 3 or 4 most distal segments.

Generally the type II setae become more recumbent distally. An exception is the occurrence of a single very long (700 to 1100 µm), recumbent seta usually on each of segments 2, 3 and 4 (Plate 23).

On the distal segment there are 13 to 15 setae. At least 4 of these appear to have tips resembling type I setae while the tips of at least 6 of the remainder resemble type II setae. Due to the presence of broken tips and difficulties with orientation under the SEM it is presently impossible to be more precise about the actual numbers of type I and type II setae on the distal segment.

(ii) *Arrangement of setae in the lateral row.* In the lateral row there are less setae/segment than in the mesial row (Table 1, cf. Plate 20 with 21). Like the mesial row, however, the type II seta(e) of each segment lie(s) dorsal to the type I seta(e) (Plate 21).

With the exception of the proximal segment the setae are grouped 40 to 100 µm from the distal end of each segment. On the proximal segment the seta are arranged in a longitudinally-orientated group which extends two-thirds of the way down the segment. Distally the length of the type I setae decreases only slightly and they remain erect along the IF (Plate 23). In contrast, the type II setae decrease in length and become increasingly recumbent so that on the 3 or 4 most distal segments they are shorter than the type I setae and are orientated almost parallel with the flagellar exoskeleton (Plate 21).

(2) Analysis of the Antennular Activities

Most movements at the joints of the antennule constitute components of four basic activities: flicking, rotation, wiping and withdrawal. Throughout these experiments, however, slow flexions and extensions at the proximal segment-medial segment (PS-MS), medial segment-distal segment (MS-DS) or the distal segment-outer flagellum (DS-OF) joints were frequently observed. These movements were not associated with the basic activities, and their frequency, extent and speed were highly variable within and between animals. An accurate description of such movements would require a highly statistical approach based on a large number of observations, and for this reason more attention was directed towards the other more stereotyped antennular activities.

(a) Antennular flicking

1. *Frequency, asymmetry and arrhythmia.*

In the holding tanks crabs flick their antennules almost continuously. Even when a crab is withdrawn into its shell flicking usually resumes long before the crab emerges.

The mean frequency of flicking appears to be influenced by the physiological condition of a crab as well as by many sensory parameters such as chemicals, light, osmotic and mechanical stimuli. Although the effects of single modalities on the mean flicking frequency have not been exhaustively tested, the following stimuli have been noted to influence the flicking frequency:

(i) *Inhibitory*. A brief interruption of flicking resulted from: light mechanical stimulation of the antennules, eyestalks or antennae; the application of distilled water to the OF; firm taps to the body or shell. Tonic decreases in the frequency of flicking resulted from: repeated application of the above stimuli; raising the water temperature above 18°C; cutting off water currents.

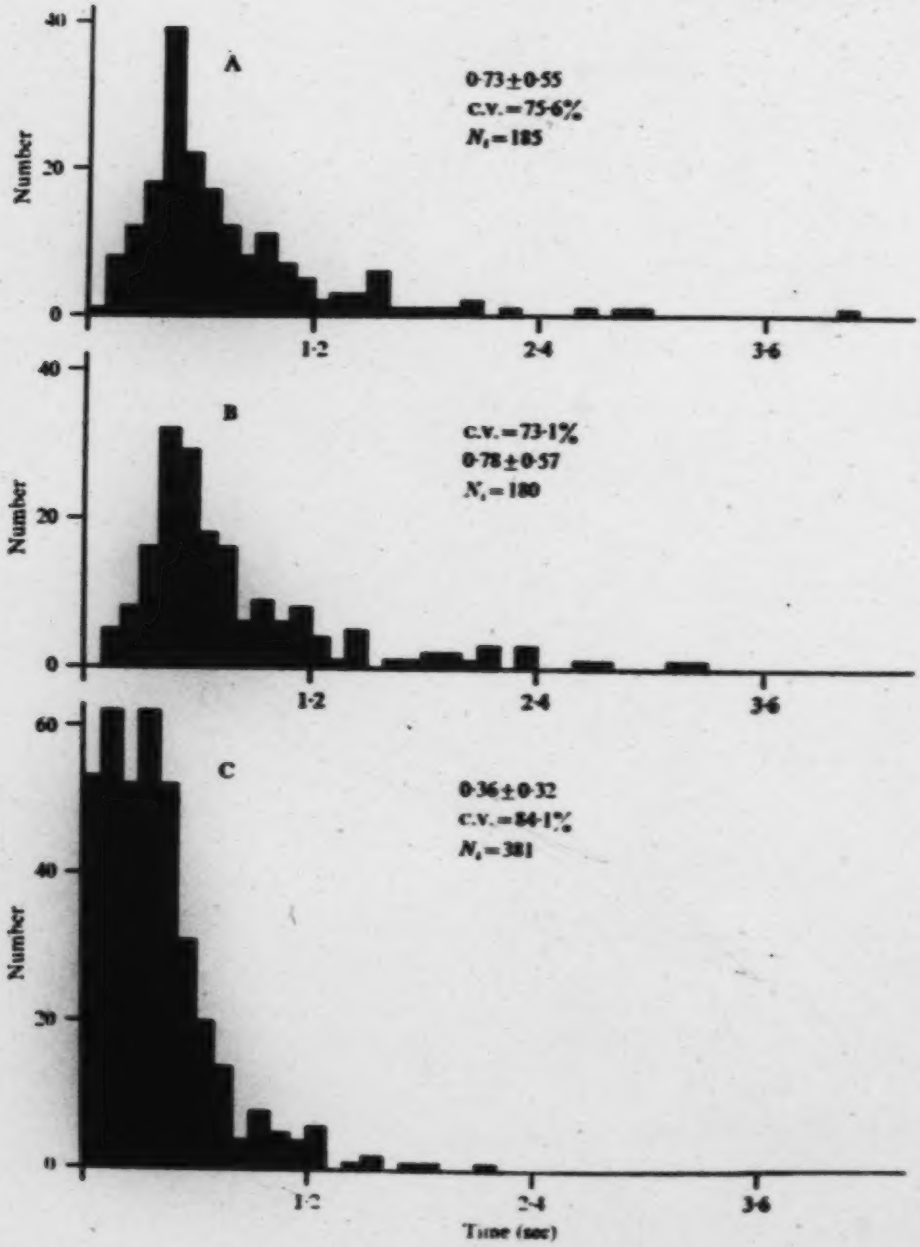
(ii) *Excitatory*. An increased frequency of flicking resulted from: faster water currents (used in high-speed filming); placing crabs in new surroundings; placing fish juice or pieces of fish near a starved animal.

Analysis of 50 frame/sec motion pictures has shown that flicking is a non-rhythmical, asymmetrical activity. The inter-flick interval of a single antennule shows large variations between successive flicks (Fig. 3A,B) and these variations do not appear to follow any simple temporal pattern. The mean frequency of flicking (1/mean inter-flick interval [min]) of a single antennule was about 75/min, under the filming conditions.

A flick of one antennule did not influence the occurrence of a flick in the other antennule. When flicks of the left and right antennules are considered together the inter-flick interval is also highly variable (Fig. 3C). The mean flicking frequency was then about 150/min under the filming conditions. Frequently animals would flick a single antennule several times without flicking the other antennule, but over the 2·5 min filming periods the numbers of flicks of each antennule were approximately the same (Fig. 3A,B). Although at 50 frames/sec flicks of the left and right antennules sometimes appeared to occur

Fig. 3. Histogram of the inter-flick interval of the left (A), the right (B) and the interval between the flick of one antennule and the next flick, whether by the ipsilateral or the contralateral antennule (C). Also shown are the mean inter-flick intervals ± standard deviations (sec), the coefficient of variation (c.v.) and the number of intervals (N_i) over a total filming period of 2·5 min. Note the high coefficients of variation. Bin size equals 0·1 sec.

(3)


A
0·73±0·55
c.v.=75·6%
N_t=185
B
c.v.=73·1%
0·78±0·57
N_t=180
C
0·36±0·32
c.v.=84·1%
N_t=381
Number
0
20
40
60
1·2
2·4
3·6
Time (sec)

synchronously, at 400 frames/sec a flick of one antennule was never exactly synchronized with a flick of the other antennule.

Flicking was not inhibited by removal of one antennule, and even after cutting an antennule off just distal to the MS-DS joint the stump of the DS was still occasionally flicked down.

2. Movements of the joints and aesthetasc setae

Antennular flicking consists of a phasic depression of the OF and DS. Analysis of high-speed motion pictures has shown that a single flick usually lasts 100 to 160 msec. Table 2 and Figure 4 show the timing of movements at the MS-DS and DS-OF joints. Some error in these measurements results from the minimum inter-frame interval of 2·5 msec (400 frames/sec) being large in comparison with some of the durations being measured.

The first evidence of a flick is a very small flexion at the MS-DS joint which usually occupies the time between two consecutive frames (2·5 msec). The second frame is considered to mark the zero time of a flick (Figs. 4A and 5A). During the second to sixth frames (10 msec) the DS-OF joint is flexed through an angle of 30 to 45° (Fig. 4A,B). From the second to fourth frame (5 msec) no further angle change occurs at the MS-DS joint, but over the next four to six frames (20 to 30 msec) this joint is flexed through 5 to 15° (Fig. 4B,C). Towards the end of MS-DS flexion there is a slight outward movement of the antennule around the PS-MS joint. The DS-OF and MS-DS joints are usually retained in their flexed positions for 20 to 35 and 5 to 11 msec, respectively. Extension at the DS-OF joint begins 30 to 45 msec after the beginning of a flick (Table 2).

Table 2. *Timing of joint movements during antennular flicking*

		DS-OF joint			MS-DS joint					
Row no.	Whole flick, $\bar{X}$±S.D./C.V.	Flexion, $\bar{X}$±S.D./C.V.	Maintained flexed, $\bar{X}$±S.D./C.V.	Extension, $\bar{X}$±S.D./C.V.	Flexion, $\bar{X}$±S.D./C.V.	Maintained flexed, $\bar{X}$±S.D./C.V.	Extension $\bar{X}$±S.D./C.V.	Initial movement of MS-DS joint to movement of DS-OF joint, $\bar{X}$±S.D./C.V.	Pause in flexion of MS-DS joint, $\bar{X}$±S.D./C.V.	N
1	157·5 ± 9·0 5·7%	10·0 ± 0·0 0·0%	24·3 ± 5·6 23·2%	123·2 ± 9·7 7·9%	30·4 ± 4·3 14·2%	7·9 ± 1·6 20·4%	95·4 ± 4·9 5·1%	2·5 ± 0·0 0·0%	4·3 ± 1·1 26·4%	7
2	124·0 ± 6·7 5·3%	10·0 ± 0·0 0·0%	22·1 ± 1·7 7·7%	92·5 ± 6·3 6·8%	21·7 ± 2·6 19·9%	6·3 ± 1·3 20·6%	77·1 ± 11·0 14·3%	2·5 ± 0·0 0·0%	5·4 ± 1·9 18·9%	6
3	156·8 ± 6·6 4·2%	10·4 ± 0·9 8·4%	25·0 ± 7·9 31·6%	122·5 ± 11·8 9·6%	31·8 ± 5·0 15·6%	8·2 ± 3·9 48·0%	91·2 ± 7·5 8·2%	2·5 ± 0·0 0·0%	6·1 ± 1·2 20·4%	7
4	155	10	20	125	27·5	10	112·5	2·5	5·0	1
5	125·0 ± 9·0 7·1%	10·8 ± 1·2 10·9%	15·8 ± 2·4 15·0%	21·7 ± 4·3 20·0%	30·9 ± 1·2 3·8%	4·2 ± 1·2 28·3%	90·8 ± 7·8 8·5%	2·5 ± 0·0 0·0%	0·0*	3
6	204·0 ± 24·1 11·8%	10·5 ± 1·0 9·5%	26·0 ± 2·0 7·7%	176·0 ± 27·7 15·7%	34·5 ± 4·3 12·5%	7·0 ± 2·5 35·0%	139·5 ± 27·4 19·7%	119·0 ± 63·3 57·4%	0·0†	5

Table shows the mean times (msec) ± standard deviations ($\bar{X}$±S.D.) and the coefficients of variation (C.V.), of the duration and patterning of antennular joint movements. Rows 1 to 3: normal flicks of the left antennule of three animals. Row 4: flick of the right antennule of animal featured in row 3. Row 5: flicks of the left antennule following removal of all but the basal segment of the OF. Row 6: flicks of the left antennule of an animal in which flicks usually followed a smooth, slow, prolonged flexion at the MS-DS joint.

*In the absence of most of the OF there is no pause in the flexion at the MS-DS joint during flexion at the DS-OF joint.

†When prolonged flexion at the MS-DS joint precedes flexion at the DS-OF joint there is no pause in the flexion of the MS-DS joint during flexion of the DS-OF joint.

Fig. 4. Movements of the MS-DS and DS-OF joints during an antennular flick. (A) shows the antennule following the small initial flexion of the MS-DS joint. During the next 10 msec the DS-OF joint is fully flexed (B). No flexion of the MS-DS joint occurs during the first 5 msec of a flick and once re-initiated lasts 30 msec (C). The dotted lines in B and C represent the position of the DS at the beginning of a flick (A). After a pause, the MS-DS and DS-OF joints are extended (D) returning the antennule to its initial posture. During the first 15 msec the aesthetasc setae are splayed apart (E).

(4)

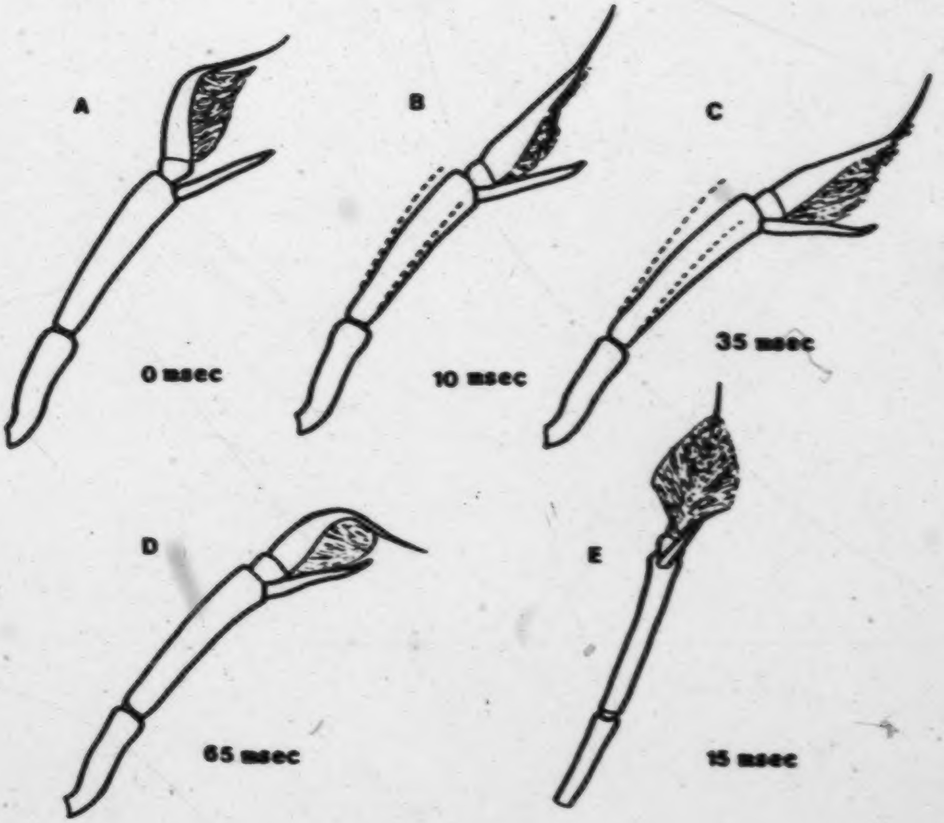
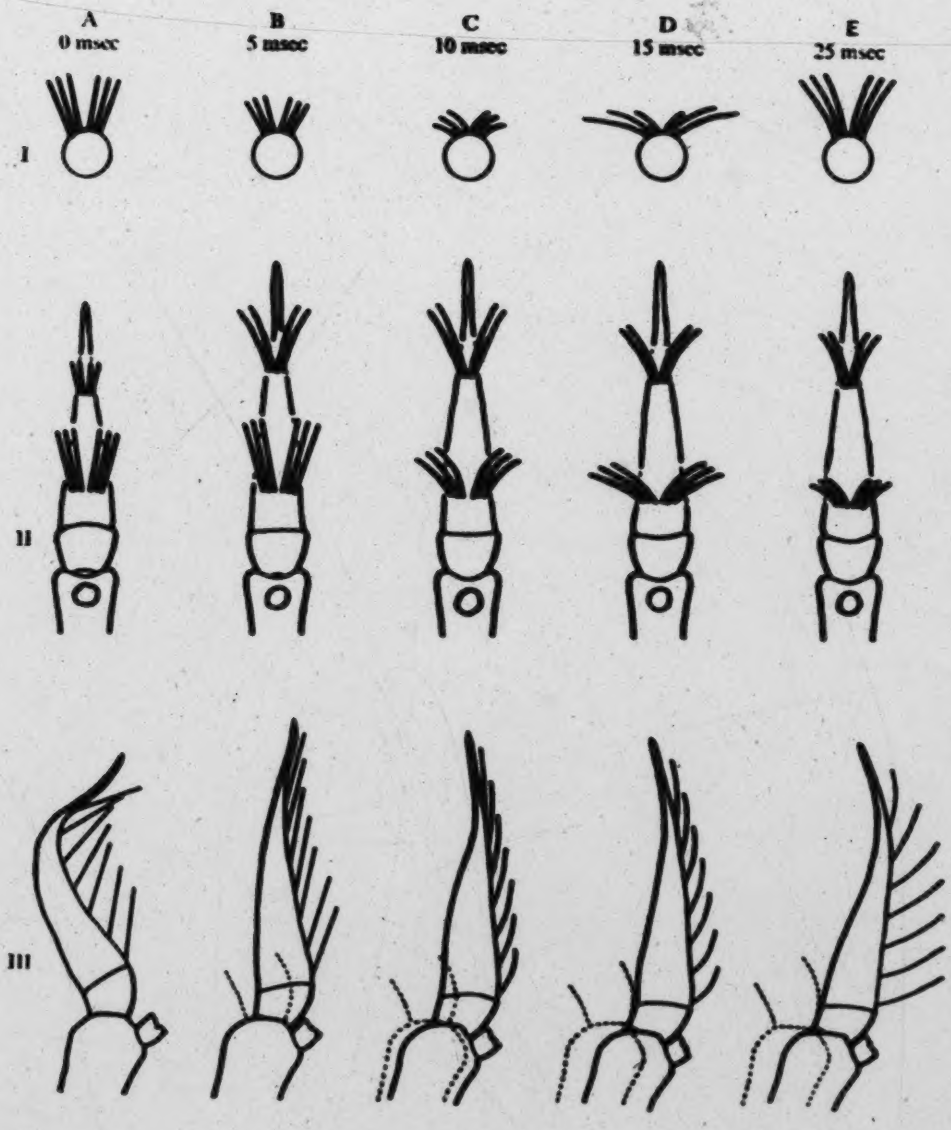

A
B
C
0 msec
10 msec
35 msec
D
E
65 msec
15 msec

Fig. 5. Diagrammatic representation of the movements of the aesthetasc setae and OF during the first 25 msec of an antennular flick. Line III shows a lateral view of the movements of the end seta in each of 7 long rows of aesthetascs. The dotted lines represent the initial position (0 msec) of the DS and OF. Note the greater separation between the more distal rows of aesthetascs and the concurrent bending of the OF (EIII). Line II shows a frontal view of the movements of the setae in row 2 and row 6 (CII). Line I shows the movements of the setae in row 2 as they might appear in a cross-sectional view of the OF. See text for further explanation.

(5)


A
0 msec
B
5 msec
C
10 msec
D
15 msec
E
25 msec
I
II
III

A feature of most movements during antennular flicking is their variability. Within any individual, however, the time required for flexion of the DS-OF joint is fairly constant (Table 2). In addition, the time between initial movement of the MS-DS and DS-OF joints appears constant at 2·5 msec, but it must be emphasized that a film speed of 400 frames/sec does not allow measurements of variability of less than 2·5 msec.

Although flicking usually occurs while the antennule is in its extended position shown in Figure 4A, it may also occur during tonic flexion at the MS-DS joint. Under these circumstances there is little or no movement at the MS-DS joint but flexion at the DS-OF joint is unaffected. This form of flicking was frequently observed in animals which had withdrawn into their shells when extension of the antennule would result in the outer flagellum hitting the inside of the shell.

Sometimes animals were encountered in which a flick was frequently preceded by a prolonged, slow flexion of the MS-DS joint. In analyzing this activity a flick was considered to begin at the first sign of depression of the OF. The initial flexion at the MS-DS joint may take up to 240 msec during which time the angle of the MS-DS joint may change up to 35°. When the flick begins there is no pause in MS-DS flexion. A flick of this type takes a longer total time (e.g. 200 msec) and examination of the various phases of a flick show that this is due to increase in the extension times of the MS-DS and DS-OF joints (Table 2).

During antennular flicking there is considerable bending of the OF and movement of the densely-packed aesthetasc setae (Fig. 4). When animals flicked towards the camera the aesthetasc setae could be seen to splay out laterally and mesially (Fig. 4E). Although the resolution on single frames was insufficient to plot the movements of single setae, observations of many flicks, slowed down about 100 times, furnished the following description. During the first 5 msec of DS-OF joint flexion the aesthetascs are depressed into a more recumbent position (Fig. 5A,B). Over the next 10 to 15 msec there is flexion at the MS-DS joint and the aesthetascs are splayed. In the resting antennule a medial part in the aesthetascs divides them into a lateral and a mesial group so that during a flick the water resistance forces generated by flexion at the DS-OF and MS-DS joints are probably sufficient to cause full splaying of the setae. Maximal splaying occurs 15 to 20 msec after the beginning of a flick (Fig. 5C,D) and is followed by movement of the setae back to their resting position over the next 30 to 40 msec (Fig. 4C). Amongst the proximal rows of setae this movement consists of a lateral or mesial swing of the setae around the OF which initially results in them returning to a less recumbent position than they adopt in the resting antennule (Fig. 5E). Lateral-mesial splaying of the aesthetascs is maximal amongst the more proximal rows of setae and it thus seems that bending of the OF facilitates a uniform separation between adjacent aesthetascs irrespective of their position on the OF (Fig. 5D,E).

During flicking, deflection of the aesthetasc setae occurs both by movement around their basal attachment with the OF and also by some bending along the shank of the setae (Fig. 5). These observations suggest that the sockets in which each aesthetasc is borne and the periodic annulations may constitute structural adaptations to antennular flicking.

The marked influence of water resistance on the OF raised the question of how much this factor was involved in the timing of movements during a normal flick. To test this, flicking was filmed in animals from which all but the basal segment of the OF had been removed. Two marked modifications were observed. First, there was no pause in the flexion of the MS-DS joint. This movement usually led flexion of the DS-OF joint by 2·5 msec (Table 2). Second, there was a reduction in the rise-time of the OF and the duration for which flexion was maintained at the MS-DS and DS-OF joints (Table 2).

The abolition of a pause in the flexion of the MS-DS joint can be explained if one assumes that in intact antennules this pause results from an upward force generated by water resistance forces to the rapid flexion at the DS-OF joint. Removal of the short segments of the OF would greatly reduce water resistance forces opposing rapid flexion at the DS-OF joint and thus the upward force on the DS during this phase of a flick. The absence of a pause following excision of the short segments cannot be explained by the decreased number of EJPs in flexor muscles 31F and 32F which results from this operation (see Section 5 *(a)*, Chapter III).

The reduction in the duration of extension at the DS-OF joint possibly results from the decrease in water resistance forces opposing the elastic forces which cause extension at the DS-OF joint. The decreased number of EJPs in flexor muscles 31F and 32F following excision of the short segments of the OF would account for the decreased times for which flexion is maintained at the MS-DS and DS-OF joints.

(b) Antennular rotation

Antennular rotation consists of a twisting of an antennule at the

PS-MS joint. The antennule can thus be rotated about its longitudinal axis through approximately 180° so that the aesthetasc setae may point back over the ipsilateral eyestalk and along the ipsilateral side of the carapace.

Usually both antennules are orientated towards the same side and both are usually re-orientated in approximate unison. Both antennules often appear to be orientated so that the aesthetascs point into existing water currents. When flicking is tonically inhibited (e.g. by removal of water currents) antennular rotation does not occur. The frequency of re-orientation is greatest when locomotory activity and the mean flicking frequency are high, as they are when the crab is placed in new surroundings. One antennule is often flicked immediately on cessation of a re-orientation movement.

(c) Antennular wiping

The specific movements employed in wiping vary between animals. The number of wiping sequences recorded from a single animal by means of high-speed filming was insufficient to confirm whether the same variation occurs within a single animal. Two sequences recorded from different animals will be detailed below as examples of this variation (Fig. 6A,B).

Sequence 1 involves slow depression of the antennule around the PS-MS joint followed by flexion at the MS-DS joint (Fig. 6AI, AII and AIII). When the DS is fully depressed the endopodites of the 3rd maxillipeds are raised and brought down along the DS to clasp the OF and IF. The PS is then raised by movements at the PS-DS and MS-DS joints and the OF and IF are thus pulled from between the endopodites

(Fig. 6AIV, AV). The antennule assumes its initial posture by further extension at the MS-DS joint (Fig. 6AV, AVI).

Sequence 2 involves earlier activation of the maxillipeds. Initial depression of the antennule occurs about its basal joint (Fig. 6BI and BII). The endopodites are brought down along the DS and clasp the OF and IF. During this phase the PS and DS are held down while the MS is elevated by angle changes around the PS-MS and MS-DS joints (Fig. 6BIII and BIV). The OF and IF are finally pulled free of the endopodites by this movement of the MS. The PS and DS are then synchronously raised about their basal joints until the antennule resumes its initial position (Fig. 6BV, BVI and BVII).

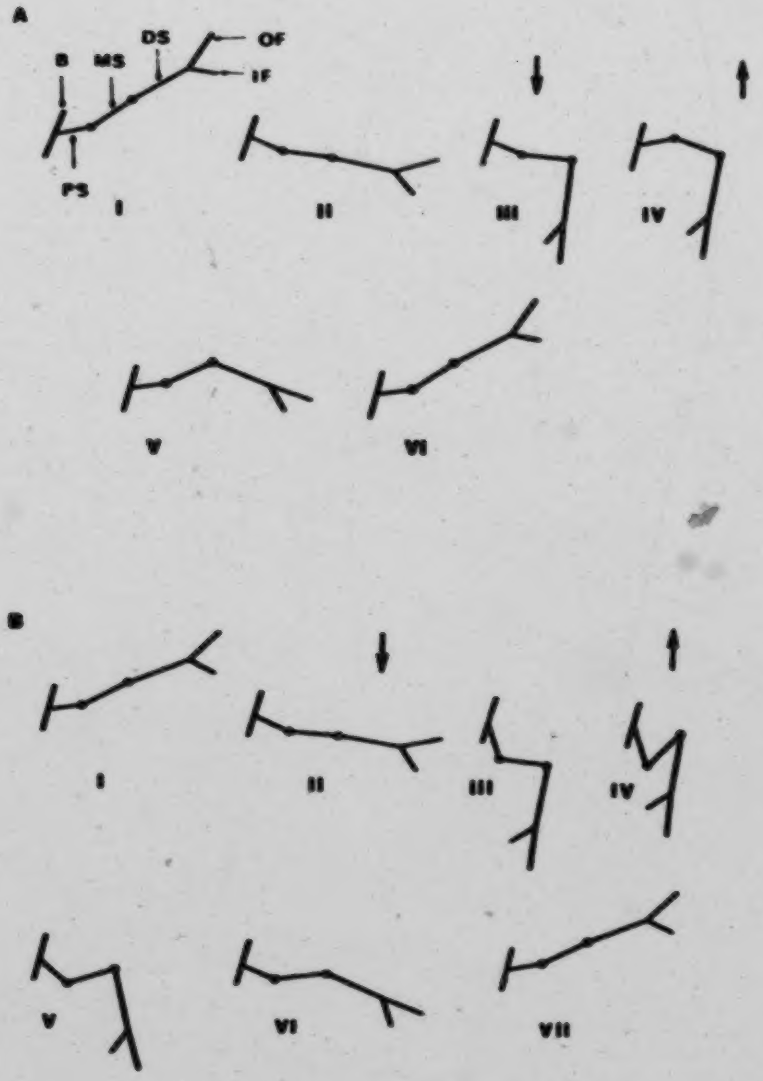
In both sequences depression of the DS is usually achieved by flexion of the MS-DS joint through 50 to 100° in 300 to 1300 msec (Fig. 6AIII and BIII). The angle moved depends on the initial angle of this joint, but the angle attained by this movement is usually between 80 and 95°. The entire wiping sequence may take from 500 to 2000 msec. From direct observations there is a strong suggestion of a slow and maintained flexion at the DS-OF joint during flexion at the MS-DS joint (Fig. 6AII, AIII, BII and BIII). Unfortunately, however, the OF is usually obscured from lateral view by the maxillipeds during this phase of wiping.

Wiping frequently occurs in the absence of apparent stimuli, although it can be elicited by light mechanical stimulation of the aesthetascs with a clean piece of filter paper. Although the interval between wipes is highly variable within any individual, an increased mean frequency of wiping has been observed in animals that had recently

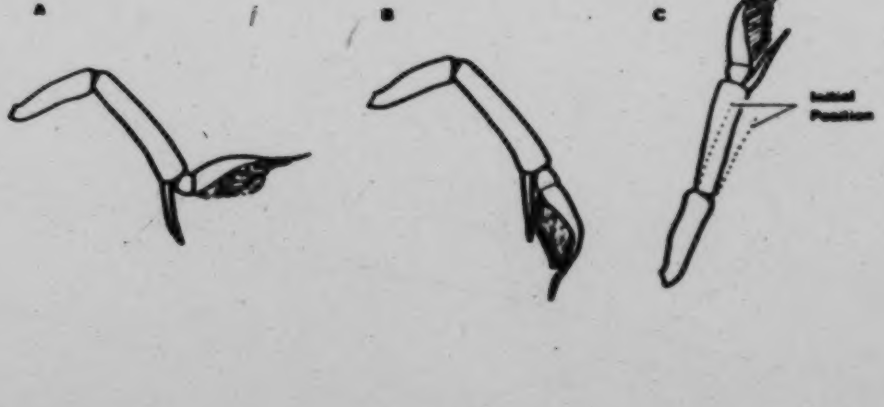
Fig. 6. Two sequences of antennular movements during wiping. The downward arrows mark the position of the antennule when the endopodites of the 3rd maxillipeds enter a sequence and the upward arrows mark where they leave a sequence. Note the variation in joint movements between sequence 1 (A) and sequence 2 (B). B, body wall; PS, proximal segment; MS, medial segment; DS, distal segment; OF, outer flagellum; IF, inner flagellum. See text for further explanation.

Fig. 7. Movements of the antennule during the withdrawal reflexes. A fast flexion-withdrawal reflex (A) involves rapid flexion at only the MS-DS joint whereas a slow flexion-withdrawal reflex (B) involves slow flexion at both the MS-DS and DS-OF joints. An extension-withdrawal reflex (C) involves extension at the MS-DS joint during which slight flexion at the DS-OF joint probably results from water resistance forces. The dotted lines in C represent the position of the distal segment prior to extension withdrawal.

(6)


A
DS
OF
B
MS
IF
PS
I
II
III
IV
V
VI
B
I
II
III
IV
V
VI
VII

(7)


A
B
C
Initial
Position

sustained damage to an OF or IF and in animals that had had carbon particles pipetted over their aesthetascs. Pipetting filtered fish juice around the aesthetascs was generally unsuccessful in eliciting wiping.

Usually only the stimulated antennule is wiped although wiping of both antennules occurs in the stimulated and unmolested animal. When one antennule is being wiped the other may continue flicking as if unaffected by the activity of the first. Occasionally the endopodites are used to wipe an eyestalk, antenna or the flagella of the 1st, 2nd and 3rd maxillipeds.

The above observations suggested that antennular wiping was functionally important in cleaning the aesthetasc setae. Material caught amongst the aesthetascs during flicking could thus be removed by the large 'combe-like' setae on the endopodites of the 3rd maxillipeds. Examination of the aesthetascs of normal animals showed them to be almost free from any debris, but after removal of even one endopodite the aesthetascs became darkened within 48 h owing to the accumulation of diatoms and dirt. Operated animals made repeated attempts to wipe their antennules with the remaining endopodite, and even following bilateral removal of the endopodites the antennules were frequently depressed into the position they adopt during wiping.

(d) Antennular withdrawal

Antennular withdrawal is considered as posturing or movement of one or both antennules away from a source of stimulation. Four types of withdrawal have been observed: (1) extension withdrawal; (2) slow flexion withdrawal; (3) fast flexion withdrawal; (4) tonic flexion withdrawal (Fig. 7A,B,C and Plate 24). The first three types are reflexes while the fourth is a postural modification of the antennule and other appendages.

1. *Extension-withdrawal reflex*

It was difficult to consistently elicit extension-withdrawal reflexes for the purposes of filming and the following description is based mainly on direct observations.

Extension withdrawal may be elicited by mechanical stimulation of the IF. Pipetting distilled water near the aesthetascs for several seconds sometimes elicits extension withdrawal but only after a slow flexion-withdrawal reflex. Extension withdrawal may thus occur when the DS is depressed or when the antennule is in the normal extended position shown in Figure 4A. In the latter case an angle change of 10 to 20° occurs at the MS-DS joint (Fig. 7C).

Stimulation of one antennule usually elicits only an ipsilateral response. Extension withdrawal may occur rapidly and smoothly or in a disjointed manner. During a rapid extension withdrawal the DS-OF joint may be slightly flexed due, probably, to the effects of water resistance.

2. *Slow flexion-withdrawal reflex*

Touching the sides or dorsal surface of the OF with a glass rod usually causes a slow, smooth flexion at both the MS-DS and DS-OF joints (Fig. 7B). Slow pipetting of diluted sea water (20%) around the aesthetascs usually produces similar movements, of one or both antennules. No withdrawal occurred when sea water was substituted in these

Plate 24. Tonic withdrawal of the antennules in a crab occupying a shell too small to permit its full withdrawal. Note the flexion of the antennules at the MS-DS and DS-OF joint, the anteriorly depressed eyestalks and the folding of the 2nd antennal segment (arrow) beneath the eyestalks and antennules.

24

experiments. Touching an eyestalk, antenna or the carapace with a glass rod elicited slow flexion withdrawal of both antennules, but a larger movement occurred on the stimulated side. The extent and rate of the component movements of slow flexion withdrawal seemed to depend on the duration or intensity of the stimulus. Very intense stimuli elicited a rapid flexion at the MS-DS joint without flexion at the DS-OF joint, but this is considered to constitute a fast flexion-withdrawal reflex.

Following a slow flexion withdrawal the antennule was normally maintained, for at least half a second, in its flexed condition. Extension from this position occurred at a variable but slow rate. The antennule was not always completely extended before the resumption of antennular flicking, and further stimulation often causes further flexion withdrawal.

3. *Fast flexion-withdrawal reflex*

The fast flexion-withdrawal reflex is most easily elicited by pipetting distilled water around the aesthetascs, strong taps to the eyestalks or shell, or bending the tip of the OF towards the aesthetascs with a glass rod. It consists of a rapid flexion of the MS-DS joint which is not accompanied by flexion of the DS-OF joint (Fig. 7A). Water resistance thus causes the OF to trail through the water in an extremely extended position. The application of these stimuli during a slow flexion withdrawal still elicits a strong flexion at the MS-DS joint characteristic of the fast flexion withdrawal.

The extent and duration of a fast flexion-withdrawal reflex varies and in part depends on the initial angle at the MS-DS joint. Usually this joint angle changes 50 to 80° in 60 to 200 msec. Extension of the antennule occurs at a variable rate and the MS-DS joint may remain flexed for several seconds.

Although mechanical stimulation of the OF results in an ipsilateral response, very intense stimuli sometimes produced a bilateral response. Generally tapping on an eyestalk or the shell or stimulation of the aesthetascs with distilled water were more effective in eliciting a response in both antennules.

4. Tonic flexion withdrawal

Animals which have been removed from their shells or animals occupying shells too small to permit their withdrawal, responded to continual tapping of the eyestalks, antennules or antennae or stimulation of the antennules with distilled water or to several hard taps to the shell or legs by adopting a tonic withdrawal posture. During tonic withdrawal the MS-DS and DS-OF joints of both antennules are flexed, the eyestalks are depressed in an anterior direction and the 2nd antennal segments are folded under the eyestalks and antennules (Plate 24). The intensity and duration of stimulation required to elicit tonic flexion withdrawal varies; animals which have recently sustained damage to an OF often showed tonic withdrawal in response to very light mechanical stimulation. Tonic withdrawal cannot be properly observed in animals which are able to withdraw completely into their shells.

(3) Motor Innervation and Musculature

(a) Anatomy

1. Muscles

The DS and MS of the antennule contain a total of five muscles which are divided into three muscle groups. Muscle group 30 consists of a single muscle, 30 (M30). Proximally the fibres of M30 are attached to the dorsal exoskeleton at the base of the MS. Distally they are attached to a tendon which is attached to the dorsal exoskeleton at the base of the DS (Fig. 1). Tension in M30 causes extension at the MS-DS joint. Cutting muscle groups 30 and 31 shows that elasticity in this joint opposes displacement of the DS from its resting position shown in Figure 1. This elasticity is weak in recently moulted animals.

Muscle group 31 consists of two muscles. Sarcomere measurements, excitatory junction potential (EJP) shapes and whole-muscle tension responses have suggested that these muscles probably contain only slow (muscle 31S) and fast (muscle 31F) fibres, respectively. Distally the fibres of both muscles share a common tendon which attaches to the ventral exoskeleton at the base of the DS. The fibres of muscle 31S (M31S) lie along a diagonal to the MS and are attached proximally to the mesial exoskeleton near the base of this segment. The fibres of muscle 31F (M31F) lie along the ventral exoskeleton of the MS to which they are attached in the proximal region (Fig. 1). Although tension in either M31S or M31F causes flexion of the MS-DS joint, the movement caused by M31F is small compared to that caused by M31S.

Muscle group 32 consists of two muscles. Sarcomere measurements, EJP shapes and whole-muscle tension responses have suggested that these muscles probably contain only slow (muscle 32S) and fast (muscle 32F) fibres, respectively. Distally the fibres of both muscles share a common tendon which attaches to the ventral exoskeleton at the base of the OF. The fibres of both muscles lie along a diagonal to the DS.

Proximally, the fibres of muscle 32S (M32S) are attached to the mesial exoskeleton about one-half to two-thirds of the way from the base of the DS, while those of muscle 32F (M32F) are attached to the mesial exoskeleton near the base of the DS (Fig. 1). Although tension in either M32S or M32F causes flexion at the DS-OF joint, M32S consists of only about six fibres and it can thus develop very little tension in comparison with M32F. When muscle group 32 is removed the OF assumes a raised position to which it returns after manual depression suggesting that elasticity within its joint with the DS is important in raising the OF during normal activity.

The five muscles described above may be grouped on the basis of their coloration and sarcomere lengths. Muscles 31F and 32F have an orange colouration while muscles 30, 31S and 32S are relatively colourless. Data from eight antennules showed that muscles 31F and 32F had short sarcomeres (2·1 to 3·9 μm), while the sarcomeres of muscles 30, 31S and 32S were about twice as long (usually 4·8 to 6·6 μm) (Table 3).

2. *Nerves*

All nerves to the OF, IF, DS and MS branch from a single nerve which enters the base of the PS. Within the base of this segment three major branches arise which will be referred to in order of decreasing diameter as nerves 1 to 3 (Fig. 1). Nerve 1 (N1) is about 280 μm in diameter and contains mainly the fine axons of the sensory neurons of the OF. Nerve 3 (N3) divides into two branches which appear to terminate near the MS-DS and DS-OF joints, respectively. Nerve 3 varies considerably in diameter between animals. No contractions or junction potentials were observed in any of the above muscles on stimulation of

Table 3. *Mean sarcomere lengths (μm) of antennular muscles*

Antennule no.	Muscle 30	Muscle 31F	Muscle 31S	Muscle 32F	Muscle 32S
1	6·1	3·7	5·6	2·4	6·6
2	4·8	3·0	5·7	2·2	6·1
3	6·4	2·5	5·6	2·6	5·6
4	5·8	3·9	5·2	2·4	5·2
5	5·4	3·2	5·5	2·1	3·8
6	5·8	3·6	5·7	2·9	5·4
7	6·1	2·4	5·3	2·6	5·5
8	3·8	2·3	4·9	2·5	5·0

Means are based on five to ten measurements of ten sarcomeres in each muscle.

N1 or N3.

Nerve 2 (N2) is about 170 μm in diameter. It is a mixed nerve containing the motor axons to all five muscles described above and sensory axons many of which arise from receptors on the IF. Within the PS a fine nerve (approx. diameter 33 μm) branches off N2 and coils around its lateral side before entering the MS. This branch will be referred to as nerve 2a (N2a) (Fig. 1).

Thin Epon sections show that N2a contains only two axons of about 13 to 14 μm in diameter and that N2 contains two giant axons of about 40 to 42 μm in diameter (Plate 25). Serial sections of antennules show that one giant axon leaves N2 in the basal half of the MS and can be seen running between M31S and M31F. The other giant axon enters the DS where it becomes associated with muscle group 32.

(b) Physiology

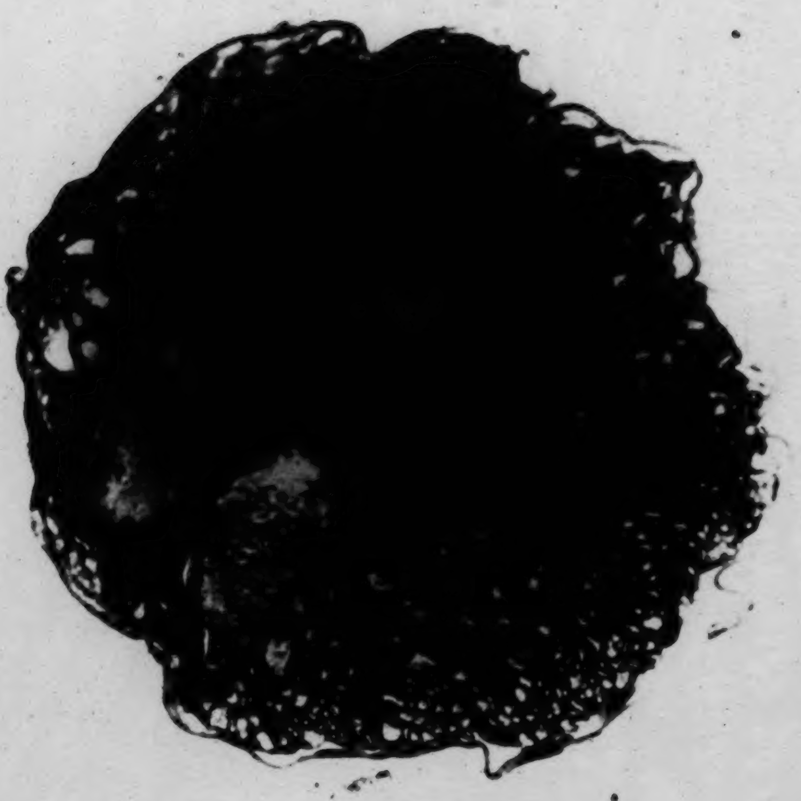
If the branch N2a is cut, stimulation of N2 evokes tension and EJPs in muscle groups 31 and 32 but not in muscle group 30. In most preparations N2 was stimulated proximally to branch N2a.

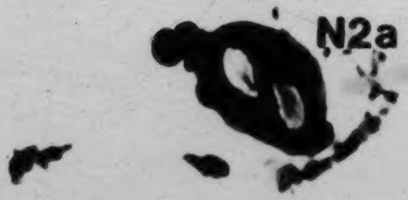
No inhibitory junction potentials were recorded in any of the muscles investigated on stimulation of any of the antennular nerves.

1. Motor innervation and tension responses of muscle group 30

Nerve 2a consists of two axons, and attempts were therefore made in each preparation to separate these axons by altering the intensity or duration of the stimulus. Two parameters were used to monitor the responses to stimulation: (1) the tension developed by M30 and (2) the occurrence of intracellularly recorded EJPs within different cells of M30. Successful separation was achieved in only 50% of these

Plate 25. A light micrograph of a cross-section of nerves 2 and 2a. Note the two axons of motoneurons A30F and A30S in nerve 2a and the giant axons of motoneurons A31F and A32F in nerve 2. X 550.





25

preparations, and examination of these records suggested that it was impossible to predict which axon would have the lower threshold. This would be expected from the similarity in diameter of the two axons (Plate 25).

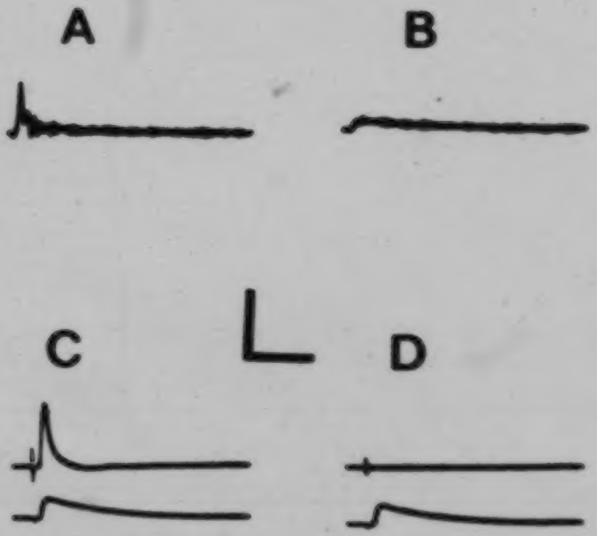
The following data were collected from preparations where the axons or motoneurons of N2a had different thresholds. When single stimuli of different intensities were applied to N2, two tension responses were elicited in muscle 30: (1) a rapid, usually larger twitch response (Fig. 8A); and (2) a slower, usually smaller contraction (Fig. 8B). The larger and smaller responses were characterized by faster and slower rates of tension development and relaxation, respectively. The relative magnitude of the larger and smaller responses varied between preparations. Even when both motoneurons were excited M30 seldom developed more than 14 mg tension.

Intracellular recording in different cells of M30 while stimulating N2 at different intensities revealed that each cell was innervated by only one motoneuron. Two microelectrodes were employed to differentiate between the EJPs evoked by each motoneuron of N2a. Although resting potentials among the fibres of M30 were usually between 50 and 60 mV, EJPs varied in size from 2 to 62 mV. It was noted that larger EJPs (39 to 62 mV) occurred only within a few fibres along the ventral surface of M30 and that elsewhere in the muscle EJPs were smaller (2 to 25 mV). The larger EJPs followed a faster time course than the smaller EJPs (Fig. 8C, 9AI and BI). Simultaneous recording in the ventral fibres and fibres elsewhere in M30 showed that the EJPs in each group of fibres had different thresholds to stimulation of N2

Fig. 8. Tension responses and intracellular recordings in muscle 30 in response to a single high-intensity and low-intensity stimulus to nerve 2. High-intensity stimulation elicits a twitch of muscle 30 (A) and an EJP in the ventral fibres (C, upper trace) and fibres elsewhere in muscle 30 (C, lower trace). Low-intensity stimulation elicits a small slow contraction of muscle 30 (B) and an EJP in fibres elsewhere in muscle 30 (D, lower trace), but no EJP in the ventral fibres (D, upper trace). Scale — A and B: 54 mg, 150 msec; C and D: 60 mV, 30 msec.

Fig. 9. AI and BI: junction potentials in the ventral fibres (large EJPs) and fibres elsewhere (smaller EJPs) in muscle 30 in response to a single high-intensity stimulus to nerve 2. AII and III and BII and III: intracellular recordings (lower two traces) and whole-muscle tension (top traces) in response to repetitive stimulation of nerve 2 at high (II) and low (III) intensity. Records A and B are from different preparations in which the motor axon to the ventral fibres (middle traces) had the highest and lowest threshold, respectively. Note the tonic tension development in the absence of a depolarization plateau (BII) (AIII). Scale — AI: 30 mV, 15 msec; BI: 15 mV, 15 msec; AII and III: 1500 msec, top traces 22 mg, middle traces 150 mV, lower traces 60 mV; BII and III: 1500 msec, top traces 54 mg, middle and lower traces 60 mV.

(8)



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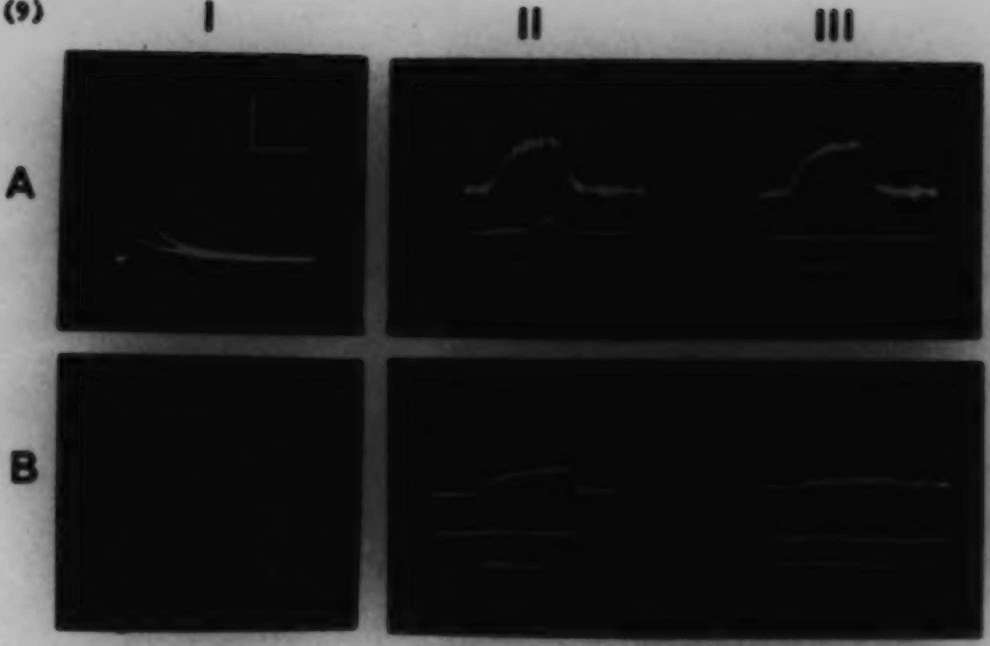


(Fig. 8C,D). It was therefore concluded that each motoneuron of N2a innervated a different population of fibres within M30. These motoneurons have been named A30F and A30S in accordance with the fast and slow time course of the tension responses they elicit in M30. Thus the motoneuron innervating the ventral fibres will be referred to as A30F while the motoneuron innervating the fibres elsewhere in the muscle will be referred to as A30S.

Repetitive stimulation of A30F at frequencies of 5, 10, 20 and 40/sec did not result in facilitation of EJPs in the ventral fibres, but stimulation of A30S at frequencies as low as 5/sec resulted in facilitation of EJPs in fibres elsewhere in M30. The degree of facilitation in the latter group of fibres was inversely related to the initial size of the EJP. Junction potentials of around 5 mV increased to three or four times their original height after four stimuli applied at 10/sec (Fig. 9AIII), yet EJPs of around 25 mV increased by only a fraction of their original size (Fig. 9BII). Junction potentials of A30F showed slight disfacilitation at frequuncies of 20/sec.

Monitoring of tension developed by M30 while simultaneously recording within fibres innervated by A30F and A30S, respectively, enabled the contractile properties of M30 to be related to its innervation. Preparations in which A30S had the lowest threshold were used to investigate the tension responses attributable to the excitation of this motoneuron, while other preparations were used to investigate responses to excitation of A30F. The stimulation of A30S at frequencies as low as 5/sec resulted in a tonic contraction of M30 on which small contractions were superimposed. Each small contraction correlated with an EJP in those muscle fibres innervated by A30S (Fig. 9AIII). Increasing the stimulus intensity resulted in an EJP in fibres innervated by A30F, and larger twitches became superimposed on the tonic contraction induced by A30S. At frequencies up to 10/sec stimulation of both motoneurons did not result in increases in the magnitude of the tonic contraction (cf. Fig. 9AII with AIII). In preparations where A30F had the lowest threshold its stimulation at frequencies up to 10/sec resulted in a series of large twitches with no tonic contraction (Fig. 9BIII). Tonic contraction, however, resulted when the stimulus intensity was above threshold for A30S (Fig. 9BII).

It should be noted that the tonic contractions observed in these experiments arose from summation of the slow 'twitches' evoked by stimulation of A30S and not by the build-up of a depolarization plateau from summation of the EJPs of this axon. The twitch response evoked by stimulation of A30F lasts for less than 50 msec whereas the decay of tonic contractions of the same magnitude may take up to 500 msec (cf. Fig. 8AIII with 9BII).

In conclusion, it appears that motoneurons A30F and A30S innervate different populations of muscle fibres. The fibres innervated by A30F are fast in character while those innervated by A30S are slow. This distinction between populations, however, is not clearly apparent from sarcomere measurements of the fibres in M30.

2. *Motor innervation and tension responses of muscle group 31*

The application of a high-intensity single stimulus to N2 evoked a large contraction (150—560 mg) in muscle group 31. This tension developed rapidly and decayed slowly. Lowering the stimulus intensity resulted in a smaller contraction (90 to 270 mg) which developed at the same rate but which decayed rapidly (Fig. 10A). Visual inspection during this twitch suggested that only M31F was contracting. After cutting M31F a single high-intensity stimulus resulted in a contraction in which tension developed at a slower rate than in the intact preparation (Fig. 10B). Maximum tension development and the rate of decay of tension were the same as in the intact preparation.

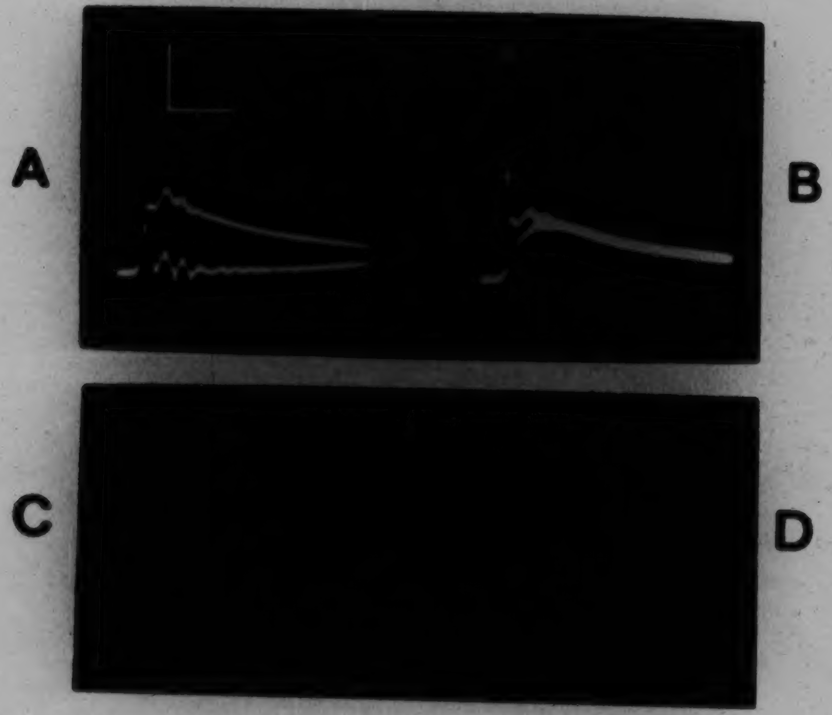
Examination of Figure 10A shows that at high intensities of stimulation tension developed rapidly up to 310 mg, while at low intensities tension developed rapidly up to 190 mg. Upon cutting M31F no rapid tension development occurred. These data suggested that M31F was responsible for rapid tension development and that it gave different responses to high-intensity and low-intensity stimulation of N2. Attempts to record these by cutting M31S were unsuccessful, probably due to damage of the motor nerve branches to M31F. In a few intact preparations, however, visual examination showed that M31S failed to respond to stimulation of N2 and the tension responses of M31F to high-intensity and low-intensity stimulation could thus be recorded. Both high-intensity (Fig. 10C) and low-intensity (Fig. 10D) stimuli evoked a twitch response. The former ranged from 200 to 400 mg in different preparations while the latter was smaller, ranging from 90 to 200 mg. These results suggest that M31F is innervated by at least two motoneurons and that stimulation of one or both of these results in small or large rapid tension development in intact preparations.

Resting potentials in cells of M31F and M31S were from 55 to 65 mV and from 40 to 70 mV, respectively. Simultaneous intracellular

Fig. 10. Tension responses of muscle group 31 in response to a single stimulus to nerve 2. A: the tension responses to a high-intensity (larger response) and a low-intensity (smaller, faster decaying response) stimulus. B: the tension responses in the same preparation to a high-intensity stimulus, before (arrow) and after cutting muscle 31F. Note the slower rate of tension development when only muscle 31S is contributing to the response. C and D: the tension responses to a high-intensity (C) and low-intensity (D) stimulus in a preparation where muscle 31S was not contracting. Note the difference in magnitude of the two responses. Owing to vibration following the twitch, records C and D have been touched by dotting through the mean points of the vibrations. Scale — 280 mg, 60 msec.

Fig. 11. Intracellular recordings in muscles 31F (top traces) and 31S (bottom traces) in response to a high-intensity (A) and low-intensity (B) stimulus to nerve 2. Note the two peaks in the top trace of A (arrow marks mechanical artifact) the second of which occurs synchronously with the peak in the lower trace. Record B shows only the EJPs of motoneurons A31F (top trace) and A31S (bottom trace). Scale — 60 mV, 15 msec.

(10)



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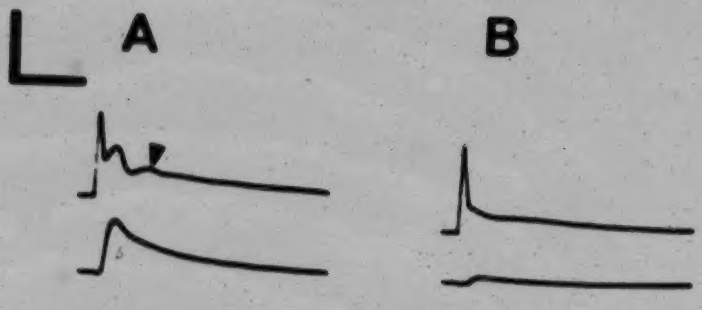


recordings in M31F and M31S during high-intensity stimulation of N2 resulted in a double-peaked response in most of the fibres of M31F and a single-peaked response in all fibres of M31S (Fig. 11A). The response in fibres of M31F consisted of a large (40 to 60 mV), sharp, initial peak which decayed rapidly but incompletely before being followed by a smaller (20 to 40 MV) blunter peak. The second peak initially decayed at about the same rate as the first but this phase was usually interrupted by movement artifacts (Fig. 11A). The first and second peaks were absent in about 5 and 20% of M31F fibres, respectively.

The electrical response in fibres M31S consisted of a blunt peak (20 to 40 mV) which had about the same magnitude and latency, in any preparation, as the second peak in fibres of M31F. Subsequent decay of the response in M31S was slow in comparison with both peaks in M31F. In all preparations the second peak in M31F had the same threshold as the response in M31S and it was thus concluded that they were EJPs of a single motoneuron which innervates both muscles. As this motoneuron may elicit slow and fast tension responses in muscle group 31 it will be referred to as motoneuron A31F-S.

Stimulation of N2 at an intensity just below the threshold of A31F-S resulted in a small EJP (1 to 5 mV) in fibres of M31S (Fig. 11B). In a few preparations no small EJPs were seen but this was probably because the axon responsible for the small EJP had a higher threshold than A31F-S in these instances. In the few fibres of M31F which did not respond to stimulation of A31F no EJP was observed on stimulation subthreshold to A31F-S. In these preparations small EJPs could be simultaneously recorded in M31S and it was therefore concluded that these resulted from excitation of a single motoneuron which innervates only M31S. Because of the low rate of decay of the EJPs of this motoneuron and the slow tension responses and long sarcomeres of M31S, this motoneuron will be referred to as A31S.

Stimulation at intensities subthreshold to A31F-S and A31S or just A31F-S evoked only the first peak in most fibres of M31F (Fig. 11B). This showed an all-or-none behaviour to further decrements of the stimulus intensity. This response was concluded to be the EJP of a single motoneuron which innervates M31F. Because of the rapid rate of decay of the EJPs of this motoneuron and the fast tension responses and short sarcomeres of M31F this motoneuron will be referred to as A31F.

Simultaneous recording of tension of muscle group 31 and EJPs within fibres of M31F and M31S showed that when only A31F was stimulated a brief (20 to 30 msec) twitch contraction, similar to that shown in Figure 10D, was elicited. Visual inspection showed that only M31F was involved in this response. At intensities just above threshold for A31S, M31S gave a small contraction which did not appear to alter the recorded tension. When M31F was cut this small contraction of M31S could be measured as ranging from 4 to 23 mg in different preparations. Simultaneous stimulation of A31S, A31F-S and A31F in intact preparations resulted in a rapidly developing, slowly decaying, large contraction of muscle group 31 (Fig. 10A).

Repetitive stimulation up to 40/sec of A31F did not result in facilitation of EJPs recorded in M31F or any tonic tension development.

Similarly, although stimulation of A31F-S at up to 40/sec did not result in facilitation of EJPs recorded in M31F or M31S, tonic tension development occurred at frequencies as low as 5/sec. This tonic tension was observed in preparations in which M31F had been severed and is due to the summation of the slowly decaying contractions of M31S (Fig. 12A). Repetitive stimulation of A31S at about 20/sec in such preparations resulted in threefold facilitation of EJPs and tonic tension development. This tonic tension was due to the summation of the small, slowly decaying contractions of M31S elicited by stimulation of A31S and not to the build-up of a depolarization plateau from summation of the EJPs of this motoneuron (Fig. 12B).

In conclusion, it appears that there are three motoneurons, A31F, A31S and A31F-S, innervating muscle group 31. Muscles 31F and 31S are composed of fibres which are fast and slow in character, respectively. The fibres of M31S are innervated by A31S. Motoneuron A31F innervates 95% of the fibres of M31F. Its low threshold suggests that A31F is the giant axon of N2 which associates with muscle group 31. Motoneuron A31F-S innervates all the fibres of M31S and 80% of the fibres of M31F.

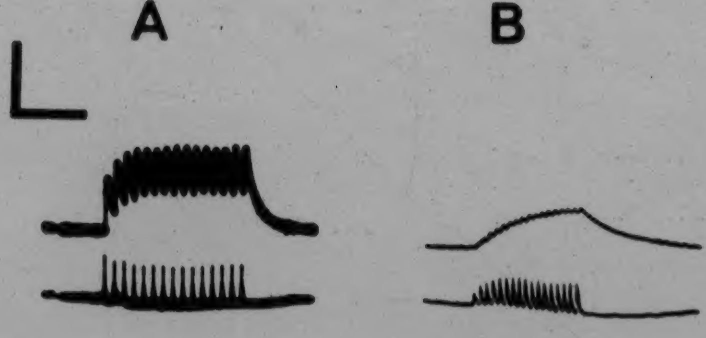
3. *Motor innervation and tension responses of muscle group 32*

The application of single stimuli to N2 elicited twitch contractions (60 to 220 mg) of muscle group 32. Careful observation of the exposed muscles showed that at a lower stimulus intensity only M32F contracted. Repetitive stimulation at frequencies up to 40/sec at this intensity resulted in a series of twitches without any tonic tension development. Increasing the stimulus intensity resulted in tonic

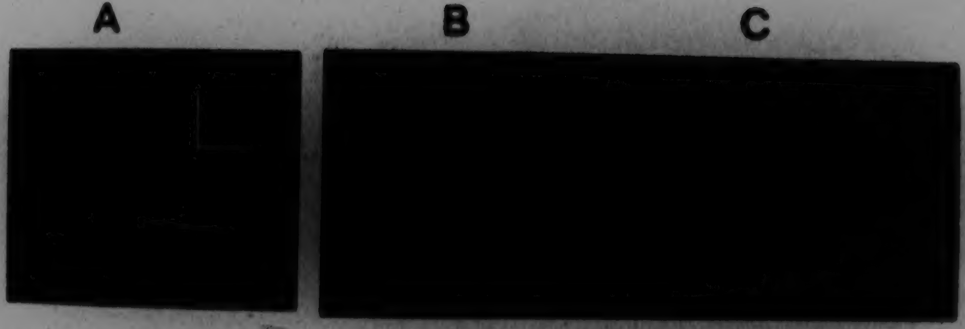
Fig. 12. Tension responses and intracellular recordings in muscle 31S during repetitive stimulation of nerve 2 at high (A) and low (B) intensity. Muscle 31F has been cut. A: high-intensity stimulation at 5/sec results in tonic tension development (top traces) due to summation of the single contractions of muscle 31S. B: low-intensity stimulation at 10/sec still results in tonic tension development (top trace) due to summation of single small contractions of muscle 31S. Scale — 104 mg, 60 mV, 1500 msec; B: 166 mg, 48 mV, 2400 msec.

Fig. 13. A: junction potentials in muscles 32F (large EJP) and 32S (smaller EJP) in response to a single high-intensity stimulus to nerve 2. B and C: tension responses in muscle group 32 (top traces) and intracellular recordings in muscles 32F (middle traces) and 32S (lower traces) in response to a high-intensity (B) and low-intensity (C) stimulus to nerve 2. Scale — A; 30 mV, 15 msec; B and C: 100 mg, 63 mV, 63 msec.

(12)



(13)



tension development at frequencies of only 10/sec. The threshold for tonic tension was identical to that for contractions in M32S and, furthermore, the rate of decay of the tonic tension was far less than that of the twitch contraction (Fig. 14A).

Simultaneous intracellular recording in M32F and M32S suggested that each muscle was innervated by a single motoneuron. The EJPs in fibres of M32F were large (40 to 72 mV) and followed a very short time course (duration: 4 msec) while those in fibres of M32S were smaller (12 to 18 mV) and followed a longer time course (duration: 50 msec) (Fig. 13A). The fibres of M32F and M32S had similar resting potentials of 58 to 75 and 60 to 70 mV, respectively.

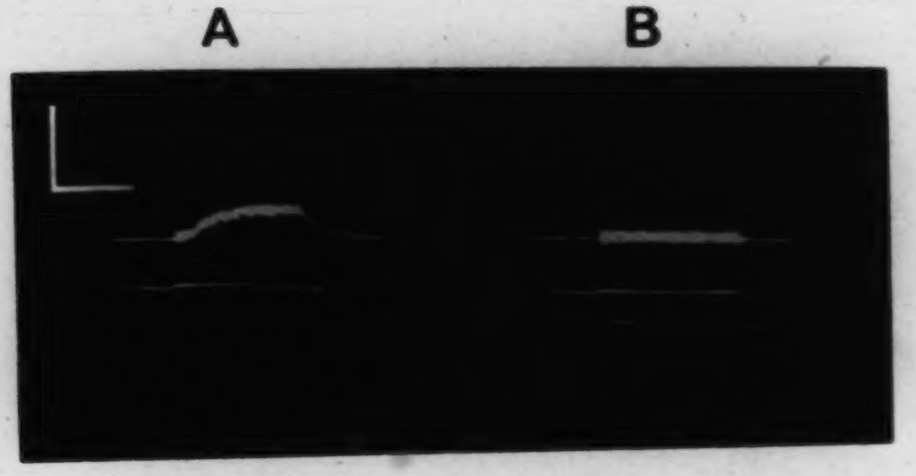
The motoneurons innervating M32F and M32S will be referred to as A32F and A32S, respectively, in accordance with the time courses of the tension responses they elicit in muscle group 32. In all preparations, A32F had a very low threshold and is therefore considered to be the giant axon in N2 which associates with muscle group 32. The marked difference in the latencies of the EJPs in M32F and in M32S further suggests that the motor axon responsible for the former is of relatively large diameter (Fig. 13A).

Simultaneous recording of tension developed by muscle group 32 and the EJPs within fibres of M32F and M32S made possible further analysis of the contractile properties of muscle group 32 in relation to its innervation. High-intensity stimulation of N2 elicited twitch contractions of muscle group 32 yet stimulating subthreshold to A32S did not elicit a detectably different contractile response (cf. Fig. 13B with 13C). Repetitive stimulation of A32F at 5 to 40/sec did not result

Fig. 14. Tension responses and intracellular recordings in muscles 32F (middle traces) and 32S (lower traces) during repetitive (10/sec) stimulation of nerve 2 at high (A) and low (B) intensity. The tonic tension development is threshold-linked to the appearance of EJPs in muscle 32S but not to the presence of a depolarization plateau. Scale — A and B: 1600 msec, top traces 110 mg, middle traces 160 mV, bottom traces 64 mV.

Fig. 15. Tension responses in muscle 32S during stimulation of nerve 2 at 5/sec (A), 10/sec (B) and 20/sec (C). Muscle 32F has been cut (see text). The tonic tension development results from summation of the single contractions of muscle 32S which can be distinguished during low-frequency stimulation (A). Scale — 54 mg, 1500 msec.

(14)



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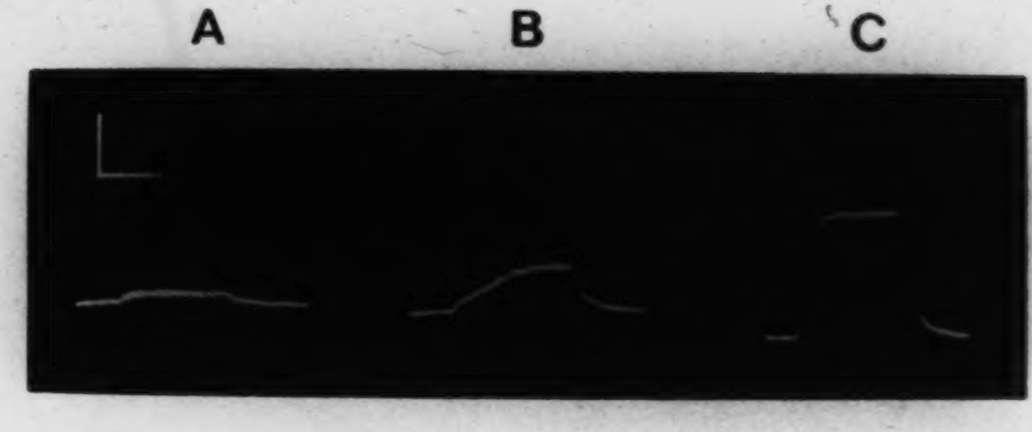


in facilitation or summation of EJPs in M32F. Disfacilitation was only very slight at frequencies from 20 to 40/sec. No tonic tension was developed, and at frequencies of 10 to 40/sec the twitch magnitude decreased by 10 to 30% over the first few twitches (Fig. 14B). Repetitive stimulation of A32S at 5 to 10/sec elicited two- to threefold facilitation of EJPs in M32S (Fig. 14A). At frequencies of 10/sec this was coupled with tonic tension development by muscle group 32, but no depolarization plateau developed in the fibres of M32S until A32S was stimulated at 20/sec. Tonic tension below this frequency can only be explained by the summation of small contractions in M32S each of which arose from a single stimulus to the motor nerve.

In order to record the contractile responses elicited by A32S alone, M32F was cut and A32F was selectively stimulated at 100/sec until the remnants of M32F ceased to respond to stimulation (90 sec). A single stimulus to N2 at just above-threshold intensity to A32S elicited a contraction in M32S. Such contractions were too small in magnitude (approx. 1 mg) to be accurately monitored on the recording system and therefore did not register at all during previous recordings of the twitches of M32F. Using high amplification and repetitive stimulation at frequencies of 5 to 20/sec a tonic tension development could be recorded (Fig. 15A-C). At frequencies of 5/sec the responses to each stimulus pulse can be seen superimposed on the tonic response (Fig. 15A).

In summary, the muscle fibres of M32F and M32S are fast and slow in character, respectively. All the fibres of M32F are innervated by motoneuron A32F while all those of M32S are innervated by motoneuron A32S.

(4) Patterns of Activity in the Antennular Motoneurons

(a) Identification of motoneurons active during specific antennular activities

Myogram records from M30 revealed both initially small, facilitating EJPs and larger, non-facilitating EJPs. The facilitating EJPs occurred at highest frequency during extension movements at the MS-DS joint (Fig. 16B) or when this joint was being held in a fully-extended position. In contrast, a burst of 1 to 4 large, non-facilitating EJPs occurred only during antennular flicks (Figs. 16A, 17A and 20).

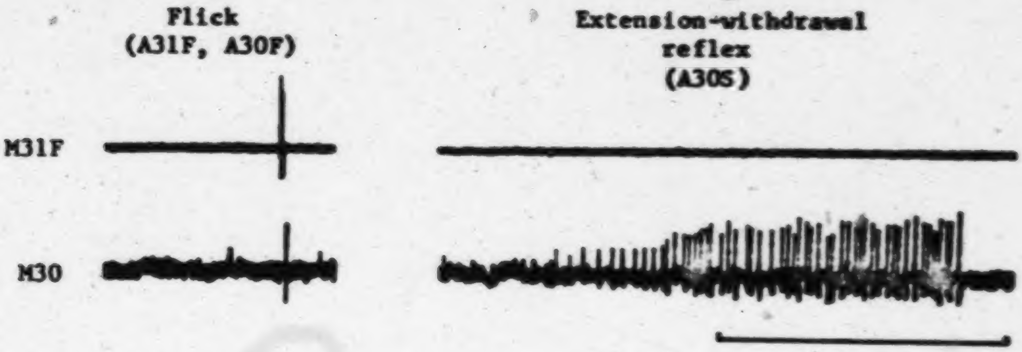
The facilitating EJPs almost certainly result from activity in motoneuron A30S which innervates the slow fibres of M30. Similarly, the non-facilitating EJPs almost certainly result from activity in motoneuron A30F which innervates the fast fibres of M30.

The synchronous recording of myograms in M31F and M31S revealed the presence of three motoneurons innervating muscle group 31. During flicking there was a burst of usually 1 to 7 large, non-facilitating EJPs in M31F but no temporally related activity in M31S (Figs. 17A, 20 and 21). The size of the EJPs usually decreased throughout these bursts but increased if an unusually long inter-EJP interval occurred (Fig. 21). In contrast, during slow or tonic flexion at the MS-DS joint, there was a train of initially small, facilitating EJPs in M31S but no activity in M31F (Fig. 18A). The non-facilitating EJPs in fast muscle M31F almost certainly represent activity in motoneuron A31F, whereas the facilitating EJPs in slow muscle M31S can be attributed to activity in motoneuron A31S.

Fig. 16. Simultaneous electromyogram recordings in flexor muscle 31F and extensor muscle 30 of the medial segment. A: recording during a flick. B: recording during an extension-withdrawal reflex. Motoneurons considered responsible for the electromyogram patterns are shown in parentheses at the top of records A and B. Time scale — 1000 msec.

Fig. 17. Simultaneous electromyogram recordings in flexor muscles 31F and 31S and extensor muscle 30. A: recording during a flick. Arrows mark pick-up of spikes in muscle 31F by the electrode in muscle 31S. B: recording during a flexion-withdrawal reflex. Arrows mark times of occurrence of spikes in motoneuron A31F-S. Motoneurons considered responsible for the electromyogram patterns are shown in parentheses at the top of records A and B. Time scale — 50 msec.

(16)


A
Flick
(A31F, A30F)
B
Extension-withdrawal
reflex
(A30S)
M31F
M30

(17)

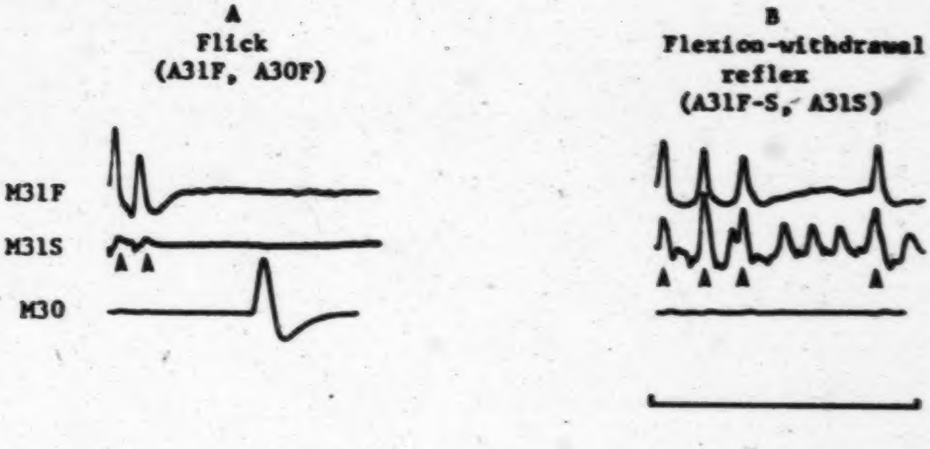
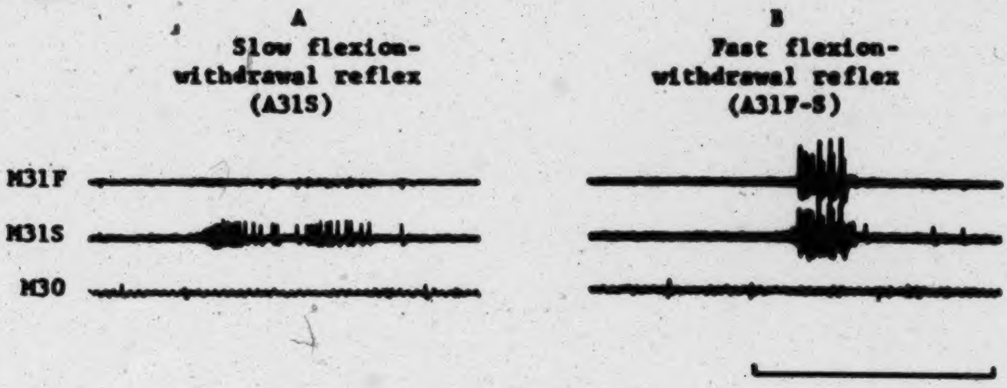

A
Flick
(A31F, A30F)
B
Flexion-withdrawal
reflex
(A31F-S, A31S)
M31F
M31S
M30

Fig. 18. Simultaneous electromyogram recordings in flexor muscles 31F and 31S and extensor muscle 30. A: recording during a slow flexion-withdrawal reflex. B: recording during a fast flexion-withdrawal reflex. Motoneurons considered responsible for the electromyogram patterns are shown in parentheses at the top of records A and B. Time scale — 1000 msec.

Fig. 19. Simultaneous electromyogram recordings in flexor muscles 31F, 31S and 32S and extensor muscle 30. During the periods of electrical activity in muscles 31S and 32S there was slow flexion at the MS-DS and DS-OF joints. During the periods of electrical activity in muscle 30 there was extension at these joints. The motoneurons considered responsible for various portions of the recording are shown in parentheses. Time scale — 1000 msec.

(18)



A
Slow flexion-withdrawal reflex
(A31S)

B
Fast flexion-withdrawal reflex
(A31F-S)

(19)

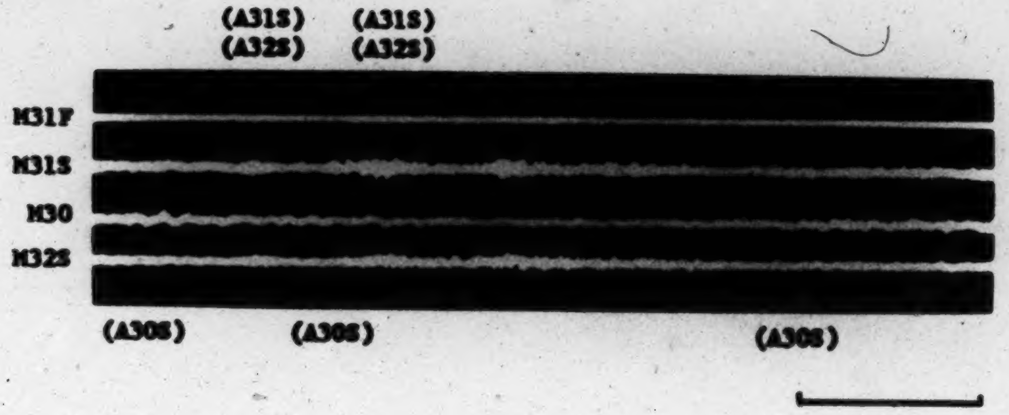
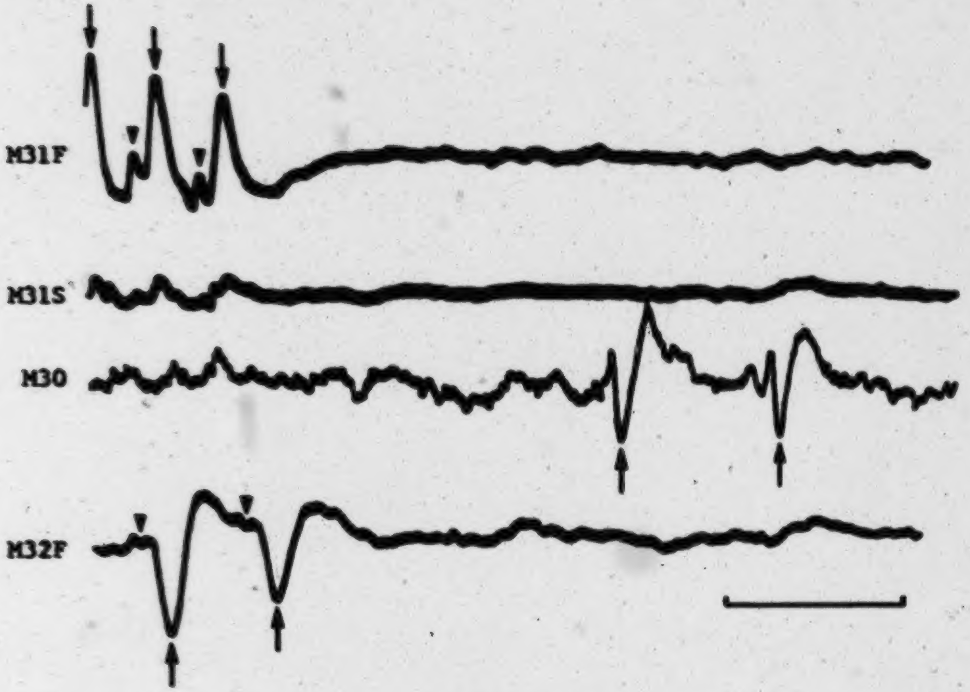


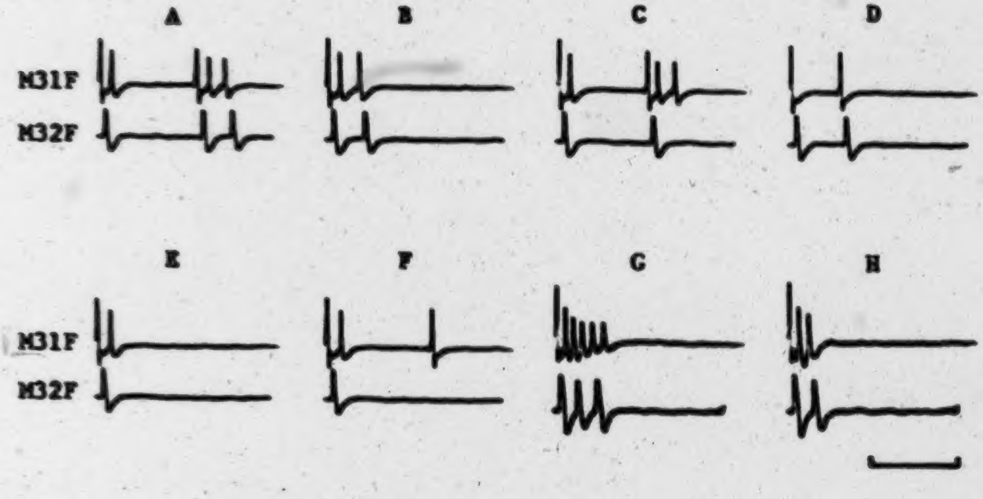
Fig. 20. Simultaneous electromyogram recordings in flexor muscles 31F, 31S and 32F and extensor muscle 30 during a single flick. The triangles mark the motoneuron spikes which were discernible in muscles 31F and 32F. Arrows mark the peaks of the EJPs which were considered to represent the time of occurrence of the spikes in motoneurons A31F (top trace), A30F (2nd bottom trace) and A32F (bottom trace). Time scale — 10 msec.

Fig. 21. A-H: simultaneous electromyogram recordings in flexor muscles 31F and 32F during single flicks in a single crab. The activity in each muscle during each flick is considered to represent a single burst. Note the extreme variation in the number of EJPs/burst, the burst lengths and the inter-EJP intervals. Note also the occurrence of bursts with many EJPs but no long inter-EJP intervals (G) and the occurrence of a very long inter-EJP interval in muscle 31F which is not followed by an EJP in muscle 32F (F). Time scale — 50 msec.

(20)



(21)



During rapid, large flexions at the MS-DS joint, a burst of large, non-facilitating EJPs occurred in both M31F and M31S. Each EJP in M31F occurred synchronously with an EJP in M31S (Figs. 17B and 18B). This myogram pattern is almost certainly the result of activity in motoneuron A31F-S which innervates both M31F and M31S.

A single electrode was used to record from muscle group 32. During flicking a burst of usually 1 to 5 large non-facilitating EJPs was observed (Figs. 20 and 21). In two preparations initially small, facilitating EJPs were recorded during slow flexion at the DS-OF joint (Fig. 19). The small, facilitating EJPs were only observed when the recording electrode was placed within the distal one-third of the distal segment. They are thus considered to be the result of activity in motoneuron A32S which innervates the tiny slow muscle M32S. In contrast, the large, non-facilitating EJPs are considered to be the result of activity in motoneuron A32F which innervates fast muscle M32F.

In conclusion, it seems that the phasic component of the antennular motor system (motor units 30F, 31F and 32F) is active during antennular flicking. In contrast, slow or tonic flexion at the MS-DS or DS-OF joints or slow extension at the MS-DS joint is the result of activity in the tonic component of the antennular motor system (motor units 30S, 31S and 32S). Furthermore, powerful flexion movements at the MS-DS joint reflects activity in the phaso-tonic component of the antennular motor system (motor unit 31F-S).

Because of electrode displacements clear records were not obtained during antennular wiping. Nevertheless, some incomplete recordings suggest that motor units 30F, 31F and 32F are not involved in wiping. Motor unit 31F-S may be active during a wipe in addition to motor units 30S, 31S and 32S.

(b) Motoneuronal activity during antennular flicking

1. Estimation of timing of motoneuronal spikes from myogram records

During flicking there is a high-frequency (100 to 300/sec) burst of non-facilitating EJPs in M31F, M32F and M30 (Fig. 20; Table 8). A single EJP often had a duration of only 5 msec. In analysing the patterns of motoneuronal activity during flicking it was thus necessary to define a point in time during each EJP as representing the time of occurrence of a spike in motoneurons A30F, A31F or A32F. Usually the EJPs were biphasic and the peak of the first phase was chosen to represent the time of occurrence of the just presynaptic motoneuron spike (Fig. 20).

This approach makes two assumptions: (1) that the time of neuromuscular transmission and the rise-time of the extracellularly recorded EJP are constant within and between fast motor units 30F, 31F and 32F; (2) that the time of occurrence of the first peak of an EJP is not related to the position of the recording electrode along the muscle. Because of the close temporal spacing of bursts in M31F and M32F these assumptions are most critical to analysing activity in motoneurons A31F and A32F. Examining the validity of these assumptions is thus restricted to motor units 31F and 32F.

With respect to assumption (1), there is little variability in the latency of the EJP recorded in M31F or M32F on stimulation of

Table 4. *Delay between the first EJPs in flexor muscles 31F and 32F during flicking and following a single shock to the motor nerve (Nerve 2)*

	Flicks			Stimulate Nerve 2		
Preparation	$\bar{X}$ ± SD	N	CV	$\bar{X}$ ± SD	N	CV
1	4·13 ± 0·376	48	9·1%	2·02 ± 0·089	48	4.4%
2	3·08 ± 0·806	16	26·2%	1·20 ± 0·063	16	5·3%
3	3·74 ± 0·138	12	3·7%	1·29 ± 0·052	12	4·0%
4	3·64 ± 0·191	39	5·2%			
5	3·76 ± 0·252	70	6·7%			
6	2·30 ± 0·368	20	16·0%			
7	2·87 ± 0·230	52	8·0%			
8	2·75 ± 0·225	35	8·2%			

Table shows the mean times (msec) ± standard deviations ($\bar{X}$±SD), the number of observations (N) and the coefficients of variation (CV) of the delay between the first EJPs in muscles 31F and 32F.

nerve 2 (Table 4). Furthermore, when the motoneuron spike was recorded from either motor unit, the first peak of the EJP always followed it with a constant latency (see Fig. 20). This criterion, however, cannot be used for comparisons between motor units because of variations in the waveform of the extracellularly recorded motoneuron spike.

With respect to assumption (2), recording simultaneously at two different points along M31F or M32F showed that the first peak of an EJP may be shifted by up to 1·0 msec by altering the recording site. The recording sites used in most preparations are shown in Figure 2B. The recording site on both M31F and M32F usually gave the earliest first peak in dual electrode recordings from these muscles. Thus I consider that: (1) the variation in the timing of EJPs within and between motor units 31F and 32F accurately reflects variation in the time of arrival of spikes at the neuromuscular terminals of motoneurons A31F and A32F, and (2) the precise delay between just presynaptic spikes in motoneuron A31F and such spikes in motoneuron A32F may have been consistently misjudged by any differences in the time of neuromuscular transmission and EJP rise-time between motor units 31F and 32F.

Dual electrode recordings in M30 proved difficult to obtain and thus the recording site generally used cannot be confirmed as giving rise to the earliest peak following a spike in motoneuron A30F. Thus the delay between spikes in motoneuron A31F or A32F and motoneuron A30F could have been overestimated by up to 1·0 msec ± differences in the times of neuromuscular transmission and EJP rise-times between motor units 31F and 30F or 32F and 30F.

2. *Motoneuronal patterns*

It was rarely possible to achieve stable, clear recordings of activity in M31F, M32F and M30 during flicking. The usual procedure was to record in M31F and M32F or M30 and to make comparisons between preparations.

A flick is initiated by a burst of 1 to 7 EJPs in M31F (Table 6). From 2·3 to 4·2 msec after the first EJP in M31F a burst of usually 1 to 5 EJPs was always observed in M32F (Figs. 20 and 21; Tables 4 and 6). The delay between these bursts cannot be fully accounted for by the conduction time in motoneuron A32F between M31F and M32F or by differences in the time of neuromuscular transmission or EJP rise-time between motor units 31F and 32F. This follows because stimulation of nerve 2 in the proximal antennular segment above the threshold of motoneurons A31F and A32F resulted in only a 1·3 to 2·0 msec delay between the EJPs recorded in M31F and M32F (Table 4). Thus at the level of the proximal segment the first spike in motoneuron A32F follows the first spike in motoneuron A31F by 1·8 to 2·1 msec (Table 4).

From 20 to 40 msec after the first EJP in M31F, a burst of 1 to 4 non-facilitating EJPs is usually recorded in M30 (Tables 5 and 7). The delay between the first EJP in M31F and the first EJP in M32F is more constant than the delay between the first EJP in M31F and the first EJP in M30 (cf. standard deviations in Tables 4 and 5).

The number of EJPs per flick in M31F, M32F and M30 was highly variable in some preparations but quite constant in others. There were never more, and usually less, EJPs in M32F than in M31F (Table 6). The most frequently encountered M31F-M32F pattern varied in different

Table 5. *Delay between the first EJPs of flexor muscle 31F and extensor muscle 30 during flicking*

Preparation	Mean Time (msec)	±	Standard deviation	No. of Observations	Coefficient of variation
1	29·5	±	4·08	56	13·8%
2	33·0	±	4·89	53	14·8%
3	28·1	±	2·55	100	9·1%
4	25·7	±	4·38	36	17·0%
5	21·4	±	0·94	10	4·4%
6	32·8	±	1·16	10	3·5%
7	36·1	±	2·12	10	5·9%
8	37·3	±	2·01	10	5·4%

Table 6. *Patterns of activity in flexor motoneurons A31F and A32F during antennular flicking*

Anim.	1/1	2/1	2/2	3/1	3/2	3/3	4/2	4/3	4/4	5/2	5/3	5/4	6/3	6/4	6/5	7/3	7/4	Total
	No. of A31F spikes/No. of A32F spikes																	
1	2	2	28	-	1	2	-	-	-	-	-	-	-	-	-	-	-	35
2	8	25	38	-	12	-	-	-	-	-	-	-	-	-	-	-	-	83
2*	-	2	2	-	57	-	26	-	-	2	2	-	5	-	-	-	-	96
3	9	18	20	-	10	-	2	1	-	-	-	-	-	-	-	-	-	60
4	-	2	-	-	45	2	3	-	-	-	12	1	2	-	1	1	1	70
5	2	17	4	4	57	-	1	1	-	-	-	-	-	-	-	-	-	86
6	1	2	1	-	6	-	29	2	-	-	7	1	4	-	-	-	-	54
7	7	53	2	5	5	-	11	-	-	4	-	-	-	-	-	-	1	88
8	-	2	10	-	36	11	1	23	4	-	8	3	-	2	-	-	-	100

*Denotes recordings from animal 2 taken 20 h after first set of recordings.

Table 7. *Patterns of activity in flexor motoneuron A31F and extensor motoneuron A30F during antennular flicking*

	No. of A31F spikes/No. of A30F spikes																	
Anim.	1/0	2/0	3/0	4/0	1/1	2/1	3/1	4/1	2/2	3/2	4/2	5/2	2/3	3/3	4/3	3/4	4/4	5/4	Total
1	1	12	2	1	4	115	36	4	24	21	6	2	-	9	2	-	1	2	243
2	-	4	-	-	2	25	1	-	21	1	-	-	2	-	-	-	-	-	60
3	62	52	5	-	14	16	6	-	-	-	3	-	-	-	-	-	-	-	155
4	-	-	-	-	-	55	6	-	27	10	3	-	-	-	-	-	-	-	101
5	-	-	-	-	-	26	-	-	4	2	-	-	-	-	-	-	-	-	33
6	5	26	1	1	2	53	15	11	3	1	2	2	-	2	-	1	1	-	126

animals. Furthermore, during long (over 10 h) recording sessions from a single animal, the most frequently occurring pattern has been observed to change (Table 6).

No clear relationship between the number of M30 EJPs and the number of M31F EJPs could be found (Table 7), but there were very rarely more and usually less EJPs in M30 than in M31F. The delay between the first EJP in M31F and the first EJP in M30 did not bear any relationship to the number of M31F EJPs.

During flicking the most frequent inter-EJP interval in M31F was in the range of 3·3 to 5·5 msec. This is less than the most frequent inter-EJP interval in M32F which was between 5·0 and 8·0 msec. When more than one EJP was recorded in M30 the most frequent interval was between 5·0 and 11·0 msec. It should be noted, however, that in a few preparations the most frequently encountered intervals in some or all of the phasic motor units lay outside these ranges.

Bursts of 4 to 6 EJPs in M31F usually contained one or more inter-EJP intervals of more than 6·0 msec. Occasionally these longer intervals also occurred in M31F bursts of only 3 and even 2 EJPs. Following a longer interval the first EJP in M31F is usually followed by 1 to 2 EJPs in M32F the first of which occurs with a delay approximately equal (within 0·2 msec) to the delay between the first EJPs in M31F and M32F on initiation of a flick. Thus a longer inter-EJP interval in M31F is often reflected by a longer inter-EJP interval in M32F (cf. Figs. 21D with 21G). It should be noted that in a few preparations flexor bursts of 3 to 5 EJPs which were not interrupted by a long inter-EJP interval (Fig. 21G) were the most frequently-occurring type.

During some flicks a single M31F EJP occurs following a longer inter-EJP interval without being accompanied by an EJP in M32F. Such EJPs may occur in the middle of a burst in M31F but are more frequently the last EJP in the burst. In the latter case such an EJP has been seen to follow an interval of up to 50 msec (Fig. 21F).

Rarely long intervals in the bursts in M31F result in an overlap of activity in this muscle with activity in extensor muscle M30 (Fig. 22A). This would be expected to result in twitches in antagonistic muscles during a single flick.

During flicking the intra-burst frequency in M30, M31F and M32F was quite variable within any preparation. Much of this variation in M31F and M32F resulted from the variability in the duration of the inter-EJP intervals. In almost all preparations, however, the mean intra-burst frequency (number of EJPs-1/burst duration) in M31F exceeded that of M32F or M30 (Table 8).

In summary a flick usually results from a burst of 2 or 3 spikes in motoneuron A31F. At the level of the proximal antennular segment the first spike in A31F is considered to precede the first spike in A32F by 1·8 to 2·1 msec. Usually only 1 or 2 spikes occur in motoneuron A32F. From 20 to 40 msec after the first spike in motoneuron A31F a burst of usually 1 to 2 spikes occurs in motoneuron A30F. Within any preparation there is often considerable variation in the structure of the bursts in motoneurons A31F, A32F and A30F.

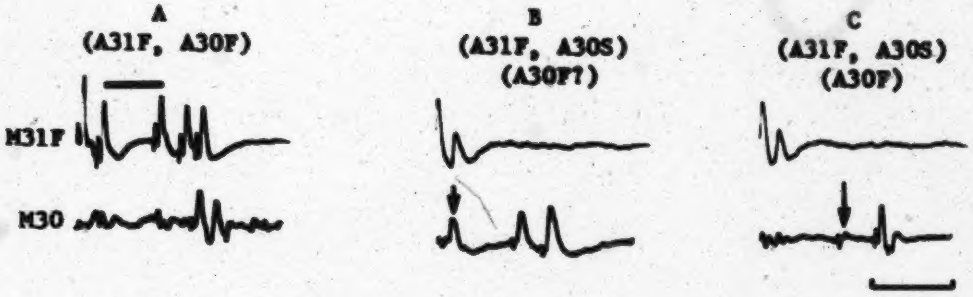
(c) Motoneuronal activity during antennular withdrawal

Bending of the tip of the OF towards the aesthetasc setae or pipetting distilled water directly over the aesthetasc setae elicited

Fig. 22. Overlap of activity in flexor muscle 31F with activity in extensor muscle 30 during flicking. A: overlap of activity in motoneuron A31F with activity in motoneuron A30F resulting from a long interval (bar) in the burst in motoneuron A31F. B and C: records from the same antennule during high- (B) and low- (C) frequency activity in motoneuron A30S. In B the first of the large, facilitated, EJPs in muscle 30 (arrow) overlaps with the burst of EJPs in muscle 31F. In comparison, C shows that during low-frequency activity in motoneuron A30S, a large EJP in muscle 30 only occurred about 28 msec after the first EJP in muscle 31F. The EJPs of motoneuron A30S (C, arrow) rarely occurred during a flick and were always of small amplitude. The motoneurons considered to be responsible for the electromyogram patterns are shown in parentheses above each record. In B it is not clear whether the third EJP in muscle 30 results from a spike in motoneuron A30F or A30S. See text for further explanation. Time scale — 20 msec.

Fig. 23. Electromyogram recordings in flexor muscle 31F and 31S and extensor muscle 30. A: low frequency of small EJPs in antagonistic muscles 31S and 30. B: stimulation of the antennule to produce extension, then slow flexion, then extension and then slow flexion movements at the MS-DS joint. Upper three traces in B are continuous with the lower three traces. Note the inhibition of high-frequency activity in one muscle before the initiation of activity in its antagonist. The motoneurons considered responsible for various portions of the recording are shown in parentheses. Time scale — A: 500 msec; B: 1000 msec.

(22)


A
(A31F, A30F)
B
(A31F, A30S)
(A30F?)
C
(A31F, A30S)
(A30F)
M31F
M30

(23)

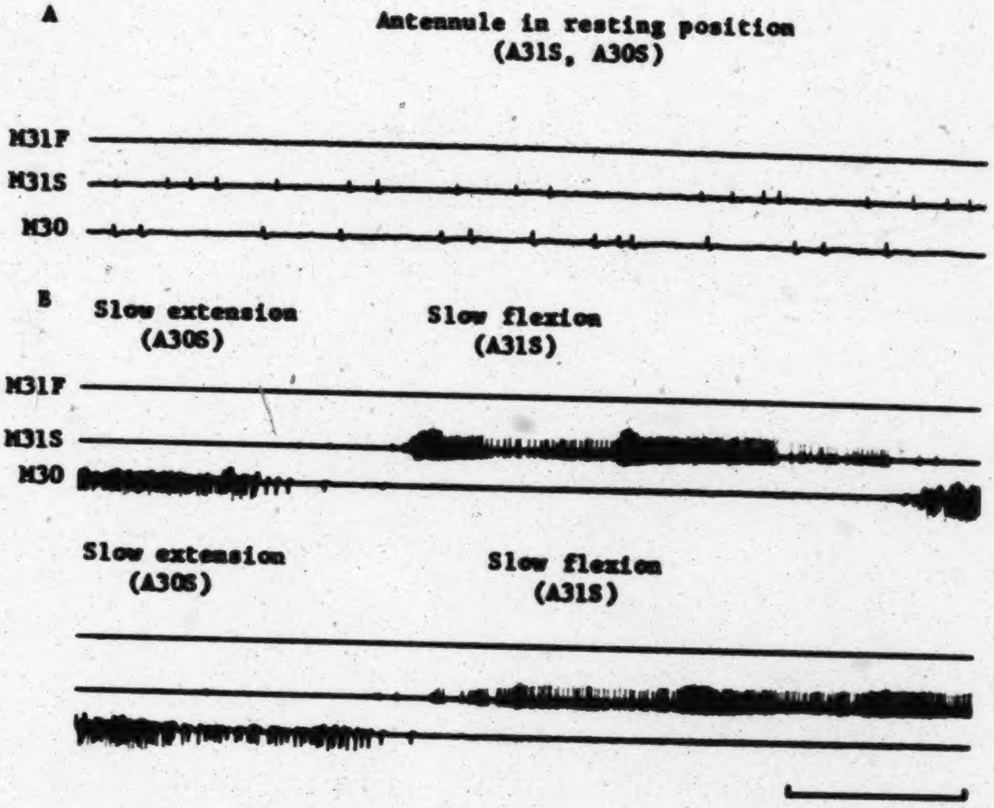

A
Antennule in resting position
(A31S, A30S)
M31F
M31S
M30
B
Slow extension
(A30S)
Slow flexion
(A31S)
M31F
M31S
M30
Slow extension
(A30S)
Slow flexion
(A31S)

Table 8. *Intra-burst frequency in motoneurons A31F, A32F and A30F during flicking*

	Motor unit 31F			Motor unit 32F			Motor unit 30F		
Animal	$\bar{f}$ ± SD	CV	N	$\bar{f}$ ± SD	CV	N	$\bar{f}$ ± SD	CV	N
1	271±11·5	4.2%	67	171±13·8	8·1%	59	———		
2	137±32·1	23·4%	52	———			96±17·8	18·2%	25
3	234±56·0	23·9%	39	199±32·8	16·5%	28	———		
3	278±12·7	4·6%	29	———			118±22·6	19·2%	25
4	272±50·9	18·7%	52	199±32·1	16·1%	51	———		
5	200±28·7	14·4%	98	———			103±29·0	28·2%	23
5	207±27·6	13·3%	47	———			209±51·5	24·6%	33
6	215±39·8	18·5%	31	249±29·9	12·0%	29	———		

Table shows the mean frequencies (no. of EJPs - 1/burst length) ($\bar{f}$), ± standard deviations (SD) and the coefficients of variation (CV), in six different animals. Where two sets of measurements are shown for a single animal, they were taken at least 1 h apart.

a burst of 1 to 16 synchronous, non-facilitating EJPs in M31F and M31S (Figs. 17B and 18B). The mean intra-burst frequency of these EJPs (number of EJPs-1/burst duration) was highly variable but has been observed to be as high as 150/sec. In two freely-moving animals such patterns were seen each time the tips of the OF touched the side of the small observation chamber. When this response was recorded the antennule was rapidly flexed at the MS-DS joint (cf. fast flexion-withdrawal reflex, Section 2 *(d)*, Chapter III) and any activity in M30 was abolished.

Bursts in motoneuron A31F-S often overlapped with activity in motoneurons A31S (Fig. 17B) and A32S. For example, when the antennule is in its resting, extended position, touching the sides or dorsal surface of the OF several times with a glass rod elicited a burst of non-facilitating EJPs in M31F and M31S which was preceded by facilitating EJPs in M31S and M32S. When this occurred extension at the MS-DS joint was usually delayed for several seconds, due, presumably, to tonic tension in M31S resulting from continued activity in motoneuron A31S.

High-frequency (15 to 60/sec) activity in motoneurons A31S and A32S (Fig. 19) and a slow flexion-withdrawal reflex could be elicited by touching the sides or dorsal surface of the OF once with a glass rod or pipetting distilled water into the sea water near the aesthetasc setae. Prolonged stimulation of the aesthetasc setae with distilled water or repetitive mechanical stimulation often resulted in high-frequency activity in motoneuron A31S being maintained for a period of minutes, even in the absence of further stimulation.

Limited observations suggest that the high-frequency activity in motoneuron A32S elicited by these stimuli is maintained for less time. During activity in motoneuron A32S the posture of the antennule was similar to that described as tonic flexion withdrawal.

Touching the IF or stroking the endopodites of the 3rd maxillipeds with a glass rod elicited an extension-withdrawal reflex and a high frequency (15 to 60/sec) of facilitating EJPs in M30 (Figs. 16B and 23B). This activity was seldom maintained for as long as activity in M31S following most flexion-withdrawal reflexes.

Continued application of distilled water to the OF often elicited high-frequency activity in motoneuron A30S but only after a period of high-frequency activity in motoneurons A31S and A32S. A most striking feature of this response was the abolition of activity in motoneurons A31S and A32S before the beginning of high-frequency activity in motoneuron A30S. Similarly, high-frequency activity in motoneurons A31S and A32S was preceded by the abolition of activity in motoneuron A30S.

In a few preparations a low frequency (mean: 7·4/sec) of small EJPs could be simultaneously recorded in M31S and M30 (Fig. 23A). During this activity the antennule was maintained in its resting position. Appropriate stimulation of the OF elicited an abolition of the small EJPs in M30 then an increase in the frequency and facilitation of the EJPs in M31S and flexion at the MS-DS joint. Stroking the endopodites of the 3rd maxillipeds during this response elicited an abolition of activity in M31S and then a high-frequency train of facilitating EJPs in M30 and extension at the MS-DS joint (Fig. 23B).

These myogram recordings clearly represent activity in motoneurons

A31S and A30S but they cannot be considered as evidence for reciprocal inhibition between these motoneurons. Firstly, the high-frequency activity in one motoneuron usually followed abolition of activity in the other motoneuron. Secondly, triggering the oscilloscope on the small EJPs in M30 or M31S, during low-frequency activity in both these muscles, did not show any period following or during an EJP in one muscle when EJPs in the other muscle were rare. Furthermore, preliminary analysis of the simultaneous, low-frequency activity in motor units 31S and 30S using cross- and autocorrelation techniques did not produce any evidence for direct, inhibitory coupling between motoneurons A31S and A30S.

In conclusion, it seems that inputs which elicit slow flexion at the MS-DS and DS-OF joints inhibit slow extensor motoneuron A30S, and that inputs which elicit extension at the MS-DS joint have a similar action on slow flexor motoneurons A31S and A32S.

(d) Overlap of activity between phasic and tonic motor units

Activity in phasic motor units 30F, 31F and 32F often overlaps with activity in tonic motor units 30S, 31S and 32S. During high-frequency activity in extensor motoneuron A30S, flicking often results in an overlapping of activity in antagonistic motoneurons A31F and A30S (Fig. 22B). Under these conditions the EJP of motoneuron A30F frequently is not recorded or cannot be recognized from the facilitated EJPs of motoneuron A30S. In the same preparation during low-frequency activity in motoneuron A30S, a burst of 1 to 4 large EJPs occurs in M30 20 to 40 msec after the first EJP in M31F (Fig. 22C). These can thus be recognized as resulting from activity in motoneuron A30F.

When flicking occurs during high-frequency activity in motoneuron A31S there is often no activity in motoneuron A30F. When motoneuron A31S is firing at frequencies above 15/sec the antennule is tonically flexed at the MS-DS joint. While recording M31F-M30 patterns during flicking, occasionally a series of flicks would occur which were not correlated with activity in motoneuron A30F. Visual examination showed that the MS-DS joint was being maintained fully flexed during these anomalous patterns. It should be noted, however, that even when the MS-DS joint was not tonically flexed, flicks occasionally occurred without activity in motoneuron A30F. Flicking usually did not occur during high-frequency activity in motoneuron A32S but it must be stressed that only 2 preparations yielded short recordings from this unit.

In conclusion it seems that high-frequency activity in slow flexor motoneuron A31S, and sometimes in slow extensor motoneuron A30S, is correlated with the abolition of activity in the fast extensor motoneuron A30F which is normally active during a flick.

(5) Central Patterning and Reflex Control of Antennular Flicking

(a) Flexor patterns following the alteration of sensory input

Flicking is not inhibited by excision of one antennule, both antennae, both eyestalks and the DS-OF joint of the remaining antennule. In such preparations the only inputs to the brain are the tegumentary nerves, the circumoesophageal connectives and the receptors in the remaining portion of one antennule. Furthermore, flicking has been observed in partially-isolated brain preparations in which only the eyestalks, antennae and antennules and their nervous connections with the brain were intact. As yet, however, flicking has not been observed in isolated brain-antennule preparations. This suggests that flicking may not occur in the total absence of non-antennular sensory input.

In intact animals a flick is initiated by a burst of 1 to 7 spikes in flexor motoneuron A31F and a burst of spikes of 1 to 5 spikes in flexor motoneuron A32F. The first spike in motoneuron A31F precedes the first spike in motoneuron A32F by an almost constant interval which in different animals lies in the range of 1·8 to 2·1 msec (Section 4 *(b)*, Chapter III). No operation to, or manipulation of, the antennules, eyestalks or antennae alters this interval.

Increasing the rate of flow of sea water through the chamber, pipetting distilled water into the inflowing stream or over the dactylopodites of the claws or legs or, in some animals, introducing fish juice to the inflowing stream, increased the mean frequency of flicking. These stimuli, however, had no effect on the number of spikes in motoneurons A31F and A32F during a single flick. Furthermore, no combination of stimuli were found which influenced the number of spikes/flick, although the most common number of spikes was sometimes seen to change during a long (over 10 h) recording session. These observations are consistent with the observation that the number of spikes in motoneurons A31F and A32F is not related to the preceding inter-flick interval. In addition, it should be noted that the percentage of long flexor bursts is quite variable between animals.

In conclusion it seems that the number of spikes in motoneurons A31F and A32F are sensitive either to subtle and slow fluctuations in the environment or to endogenous changes in the physiological state of the crab, or to both these factors. This conclusion, however, does not preclude that the number of flexor spikes is independent of any sensory input but just that any such dependency is under some form of long-term regulation.

Excision of the IF and the long, thin, distal segments of the OF, or immobilization of the MS-DS and the DS-OF joints by stapling the MS and DS to the Sylgard block (Fig. 2A) did not consistently alter the numbers of spikes in flexor motoneurons A31F and A32F (Table 9). In contrast, excision of all but the basal segment of the OF usually resulted in the almost total abolition of long bursts in motoneurons A31F and A32F (cf. Figs. 24A with Fig. 24B; Table 9). This effect was most clearly seen in crabs in which the number of spikes in motoneurons A31F and A32F was highly variable. Following this operation most flicks resulted from only 1 or 2 spikes in motoneurons A31F and A32F (Fig. 24B; Table 9). This was true even during series of flicks which had a short mean inter-flick interval. By far the most frequent pattern was 2 spikes in motoneuron A31F and 1 spike in motoneuron A32F while 1 spike in motoneuron A31F was never followed by more than 1 spike in motoneuron A32F. Records from muscles 31F and 32F more than 2 weeks after this operation showed no recovery of the ability to produce an appreciable percentage of longer bursts in motoneurons A31F and A32F. Only one animal which was initially showing a high percentage of 2:1, A31F:A32F patterns failed to show a clear reduction

Table 9. *The effects of altered sensory input on the number of spikes in flexor motoneurons A31F and A32F during antennular flicking*

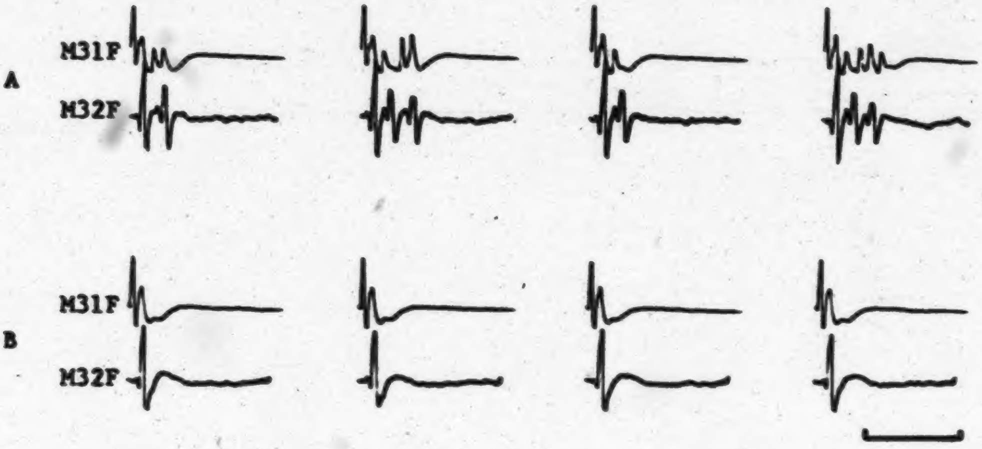
Anim.	Ant'le Cond't	No. of A31F spikes/No. of A32F spikes																			Total	% of 1/1, 2/1 & 2/2 patterns
		1/1	2/1	2/2	3/1	3/2	3/3	4/1	4/2	4/3	4/4	5/1	5/2	5/3	5/4	6/3	6/4	6/5	7/3	7/4		
1	Free	-	2	10	-	36	11	-	1	23	4	-	-	8	3	-	2	-	-	-	100	12%
1	MS-DS Im.	1	-	29	-	2	27	-	-	24	7	-	-	1	9	-	-	-	-	-	100	30%
1	MS-DS & DS-OF Im.	1	7	50	-	15	10	-	2	13	2	-	-	-	2	-	-	-	-	-	100	58%
1	DS-OF Freed	-	1	7	-	6	15	-	-	16	2	-	-	1	2	-	-	-	-	-	50	4%
1	MS-DS Freed	-	-	4	-	7	5	-	-	8	-	-	1	1	1	-	-	-	-	-	27	15%
2	Free	1	45	-	21	98	-	-	11	1	-	-	3	-	-	-	-	-	-	-	180	26%
2	MS-DS & DS-OF Im.	-	27	-	23	116	-	-	13	1	-	-	-	-	-	-	-	-	-	-	180	15%
2	OF Excised	9	145	10	-	5	-	-	1	-	-	-	-	-	-	-	-	-	-	-	170	97%
3	Free	-	2	-	-	45	2	-	3	-	-	-	-	12	1	2	-	1	1	1	70	3%
3	OF Excised	-	64	-	-	5	-	-	-	-	-	-	-	1	-	-	-	-	-	-	70	91%
4	Free	-	110	-	1	5	-	1	10	1	-	-	-	-	-	-	-	-	-	-	128	86%
4	OF Excised	6	83	16	-	11	-	-	1	-	-	-	1	-	-	-	-	-	-	-	118	89%
5	Free	9	18	20	-	10	0	0	2	1	-	-	-	-	-	-	-	-	-	-	60	78%
5	MS-DS & DS-OF Im.	-	27	8	-	12	-	-	2	2	-	-	-	3	2	-	1	-	-	-	60	58%
5	DS-OF Freed	-	20	13	-	13	5	-	1	5	1	-	-	2	-	-	-	-	-	-	60	55%
5	MS-DS Freed	1	5	17	-	9	11	-	2	3	5	-	-	6	1	-	-	-	-	-	60	38%
5	OF Excised	20	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60	100%

The patterns under each condition are those of successive flicks. In each animal operations or manipulations were imposed in the order in which they appear under antennule condition (ant'le cond't). Unless specified the antennule was always intact and unrestrained (Free). Im. - Immobilized.

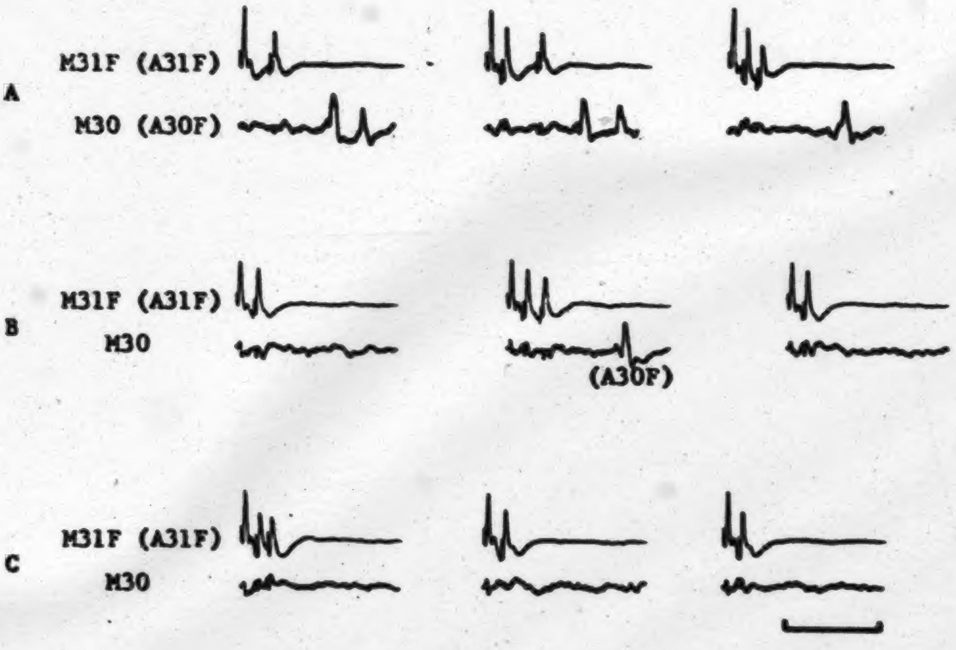
Fig. 24. Simultaneous electromyogram recordings in flexor muscles 31F and 32F during single flicks: before (A) and after (B) excision of all but the basal segment of the outer flagellum. Note the reduction in the number of EJPs in each muscle which reflects a reduction in the number of spikes in flexor motoneurons A31F and A32F. Time scale — 30 msec.

Fig. 25. Simultaneous electromyogram recordings in flexor muscle 31F and extensor muscle 30 during flicks of a free antennule (A) and following immobilization of the MS-DS joint (B) and following immobilization of both the MS-DS and DS-OF joints (C). The motoneurons considered responsible for the various patterns are shown in parentheses. Note the progressive abolition of activity in extensor motoneuron A30F through sets A, B and C. Time scale — 30 msec.

(24)


M31F
A
M32F
M31F
B
M32F

(25)


M31F (A31F)
A
M30 (A30F)
M31F (A31F)
B
M30
(A30F)
M31F (A31F)
C
M30

in the percentage of longer flexor bursts following this operation (Animal no. 4, Table 9), and in no case did this operation affect the numbers of spikes in motoneurons A31F and A32F of the contralateral antennule.

In conclusion, it appears that information from receptors on the short segments of the OF is necessary for the generation of most bursts of more than 2 spikes in flexor motoneurons A31F and A32F during flicking in intact animals.

(b) Extensor activity following the alteration of sensory input

In intact animals the extension phase of a flick is the result of a burst of 1 to 4 spikes in extensor motoneuron A30F. The first spike of this burst follows the first spike in flexor motoneuron A31F by 20 to 40 msec. The number of spikes in extensor motoneuron A30F is not related to the frequency of flicking or to the number of spikes in flexor motoneuron A31F. Furthermore, the activity in motoneuron A30F is not inhibited by the excision of the OF, IF or the DS-OF joint (Table 10).

Activity in extensor motoneuron A30F was often absent during flicks which were performed while there was tonic flexion at the MS-DS joint (Section 4 *(d)*, Chapter III). When the antennule is flicked during tonic flexion at the MS-DS joint there is little or no movement at the MS-DS joint (Section 2 *(a)*, Chapter III). This suggested that MS-DS joint flexion might be necessary for the excitation of motoneuron A30F.

To test this hypothesis the MS-DS joint was immobilized by

Table 10. *The effects of altered sensory input on the number of spikes in flexor motoneuron A31F and extensor motoneuron A30F during antennular flicks*

Anim.	Ant'le Cond't	No. of A31F spikes/No. of A30F spikes																						Total	% flicks with no A30F spk.
		1/0	2/0	3/0	4/0	5/0	6/0	1/1	2/1	3/1	4/1	5/1	6/1	2/2	3/2	4/2	5/2	2/3	3/3	4/3	3/4	4/4	5/4		
1	Free	1	12	2	1	-	-	4	115	36	4	-	-	24	21	6	2	-	-	2	1	1	2	243	7%
1	MS-DS Im.	3	75	33	7	2	1	-	7	8	8	-	-	-	1	-	-	-	-	-	-	-	-	147	82%
1	MS-DS & DS-OF Im.	6	45	11	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	67	100%
1	DS-OF Freed	-	14	8	-	-	-	-	19	10	5	2	-	-	-	-	-	-	-	-	-	-	-	58	38%
1	MS-DS Freed	-	1	-	-	-	-	-	12	2	-	-	-	19	7	-	-	-	-	5	1	1	-	48	2%
2	Free	-	-	-	-	-	-	-	26	-	-	-	-	4	2	-	-	-	1	-	-	-	-	33	0%
2	MS-DS Im.	-	2	1	-	-	-	-	10	7	-	-	-	7	1	-	-	-	-	-	-	-	-	28	11%
2	MS-DS & DS-OF Im.	-	26	2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	96%
2	DS-OF Freed	-	27	4	1	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35	91%
2	MS-DS Freed	-	-	-	-	-	-	-	16	4	-	-	-	4	2	1	-	2	4	-	-	-	-	33	0%
2	DOS-OF Excised	-	16	-	-	-	-	-	18	-	-	-	-	2	-	-	-	1	-	-	-	-	-	37	42%
3	Free	5	26	1	1	-	-	2	53	15	11	-	-	3	1	2	2	-	2	-	1	1	-	126	26%
3	MS-DS Im.	8	34	6	1	-	-	5	50	6	3	-	-	1	2	-	-	-	-	-	-	-	-	116	42%
3	MS-DS & DS-OF Im.	3	31	13	4	-	-	-	12	12	6	1	-	-	1	2	-	-	-	-	-	-	-	85	60%
3	MS-DS & DS-OF Freed	1	9	3	2	-	-	1	18	9	4	-	1	3	1	1	1	-	-	-	-	-	-	53	26%
4	Free	-	-	-	-	-	-	-	55	6	-	-	-	27	10	3	-	-	-	-	-	-	-	101	0%
4	MS-DS Im.	-	38	1	-	-	-	-	12	3	-	-	-	2	2	-	-	-	-	-	-	-	-	58	83%
4	MS-DS & DS-OF Im.	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	100%
4	DS-OF Freed	1	14	1	-	-	-	-	4	-	-	-	-	2	-	-	-	-	-	-	-	-	-	22	73%
4	MS-DS Freed	-	-	-	-	-	-	-	27	1	-	-	-	3	2	-	-	-	-	-	-	-	-	33	0%
4	MS-DS Im.	-	13	2	1	-	-	-	7	2	1	-	-	-	-	-	-	-	-	-	-	-	-	26	62%
4	MS-DS & DS-OF Im.	-	27	8	1	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	92%
4	MS-DS & DS-OF Freed	-	-	-	-	-	-	-	13	3	-	-	-	4	3	-	-	-	-	-	-	-	-	23	0%

The patterns under each condition are those of successive flicks. In each animal operations or manipulations were imposed in the order in which they appear under antennule condition (ant'le cond't). Unless specified the antennule was always intact and unrestrained (Free). Im. - Immobilized.

stapling the MS and DS to the Sylgard block. This [illegible] done in such a way that the MS-DS and DS-OF joints were in the partially extended condition they adopt in the resting antennule (Fig. 4A). In unrestrained antennules there was no activity in motoneuron A30F during 0 to 27% of flicks (Table 10). Following immobilization of the MS-DS joint 10 to 97% of flicks were not correlated with activity in motoneuron A30F (Table 10). In this condition, flicks which contained more than a single extensor spike were rare even in preparations which in the unrestrained state usually showed 2 or more extensor spikes during a flick (cf. Figs. 25A with 25B; Table 10).

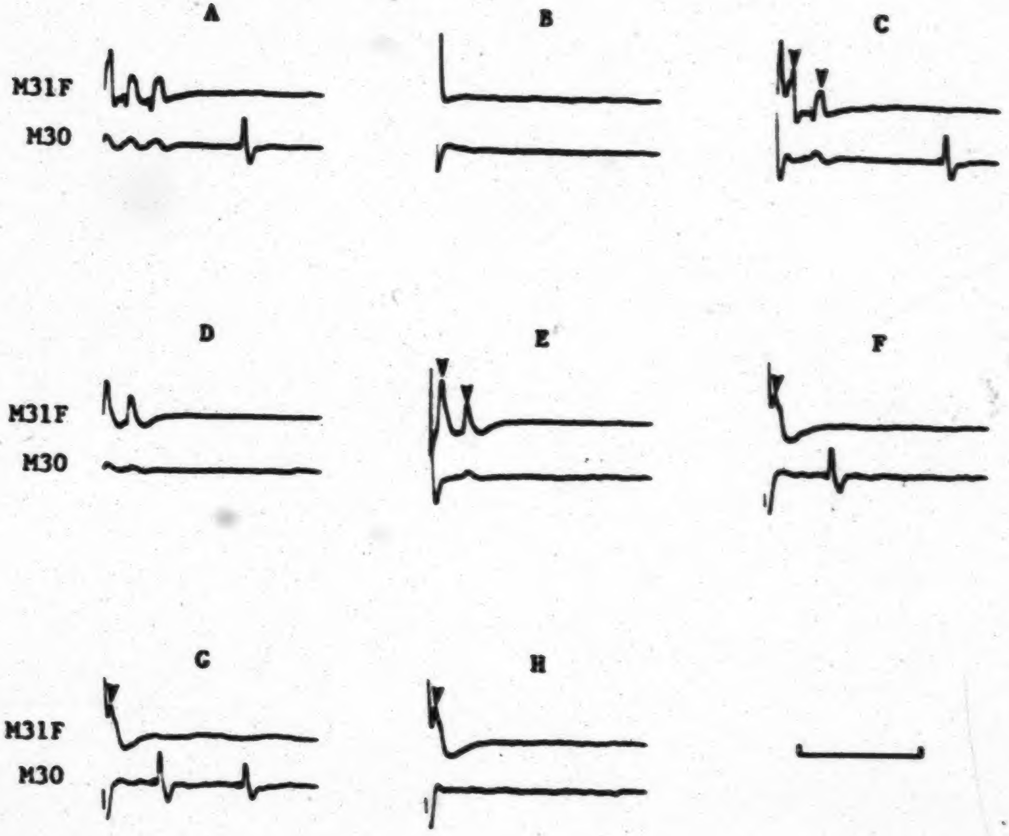
When both the MS-DS and the DS-OF joints were immobilized there was no extensor activity in 60 to 100% of flicks (Fig. 25C; Table 10). Furthermore, only 3 out of 85 flicks in a single animal were correlated with more than one extensor spike (Animal no. 3, Table 10). In this condition a flick was considered to be correlated with the appearance of bursts in flexor motoneurons A31F and A32F which were identical to those seen during flicking in an unrestrained antennule.

Releasing the DS-OF joint and then the MS-DS joint progressively restored extensor activity (Table 10). The antennule could then be immobilized and released again with qualitatively identical results (Animal no. 4, Table 10).

There was some variability between animals in the extent to which joint immobilization resulted in abolition of extensor activity. In most animals immobilization of the MS-DS and DS-OF joints abolished extensor activity in 90 to 100% of flicks. In one animal, however, only 60% of flicks were without extensor activity following complete

Fig. 26. Simultaneous electromyogram recordings in flexor muscle 31F and extensor muscle 30 of a single antennule. A: a flick of a free antennule. B: subthreshold stimulation of flexor muscle 31F. C: threshold stimulation of muscle 31F. D: a flick following immobilization of the MS-DS joint. E: threshold stimulation of flexor muscle 31F following immobilization of the MS-DS joint. F: a higher-intensity shock to flexor muscle 31F following immobilization of the MS-DS joint. G: a high-intensity shock to flexor muscle 31F in a free antennule. H: a high-intensity shock to flexor muscle 31F in a free but excised antennule. Arrows indicate spikes elicited in muscle 31F by direct stimulation. See text for further explanation. Time scale — 30 msec.

(26)



immobilization (Animal no. 3, Table 10).

In an attempt to excite motoneuron A30F by evoking flexor muscle tension and joint flexion, 2 myogram electrodes were used to simultaneously stimulate and record electrical activity in M31F, while a third electrode was used to record the EJPs of motoneuron A30F in M30. When clear records of the EJPs of motoneurons A31F and A30F were obtained (Fig. 26A), single 1 msec shocks were applied to M31F. On increasing the stimulus intensity, 1 or 2 all-or-none spikes were recorded in M31F and there was rapid flexion of the MS-DS joint (Figs. 26B and 26C). In 40% of the animals tested, up to 50% of the stimuli elicited 1 or occasionally 2 spikes in M30. The size and shape of these spikes in M30 were identical to the EJPs recorded from M30 during a flick (cf. Fig. 26A with 26C). They are thus considered to be the EJPs of motoneuron A30F. In successful preparations the EJP of motoneuron A30F followed the first spike in M31F with approximately the same delay as that separating the first EJPs in M31F and M30 during a flick (cf. Fig. 26A with 26C).

Immobilizing the MS-DS joint abolished the EJP of motoneuron A30F during most flicks and on threshold stimulation of M31F (Figs. 26D and 26E). Increasing the stimulus intensity elicited 1 or 2 large EJPs in M30 with a latency of 10 to 14 msec after the first spike in M31F (Fig. 26F). When the MS-DS joint was released a high-intensity stimulus to M31F sometimes elicited an EJP in M30 with a normal latency in addition to the short-latency EJP (Fig. 26G). The short-latency and the normal-latency EJPs were permanently abolished by excision of the antennule at the proximal segment-medial segment joint (Fig. 26H). It is thus considered that the short-latency EJP in M30 results from direct stimulation of receptor axons which have an excitatory effect on motoneuron A30F.

In excised antennules threshold stimulation of M31F always resulted in a single large spike being recorded from nerve 2. This occurred with a latency of 1·4 to 1·8 msec and was thus presumed to be an antidromically propagating action potential in motoneuron A31F. In addition, threshold stimulation of M31F elicited a multi-unit burst of spikes in nerves 2 and 3. These bursts began 10 to 15 msec after the stimulus artifact and had durations of 19 to 46 msec. Activity in some units in nerve 3 could be abolished by immobilizing the MS-DS joint but many units were still active following this manipulation.

In conclusion, it seems that flexion at the MS-DS and DS-OF joints is usually necessary for the consistent excitation of extensor motoneuron A30F during flicking. Despite this, the combined effect of antidromic activation of motoneuron A31F, tension in M31F and flexion at the MS-DS joint is only occasionally sufficient for the excitation of extensor motoneuron A30F and then only in some animals. Furthermore, antidromic activation of motoneuron A31F and tension in M31F are never sufficient to excite extensor motoneuron A30F.

IV. DISCUSSION

(1) Surface Structures of the Antennular Flagella

(a) Exoskeletal Pits and Pores

Exoskeletal pits are well distributed over the surface of the inner and outer flagella and the distal antennular segment. They are extremely tiny and in no case appeared to contain any material. Thus rather than sensory structures they are tentatively considered to be the openings of the tegumentary gland ducts (Dennell, 1961).

Exoskeletal pores identical to those described on the antennular flagella of *Pagurus alaskensis* have not been described in other crustaceans. Ong (1968) has described receptors on the mandibles of copepods which consist of a pore in the exoskeleton in which are the tips of the dendrites of 1 to 5 sensory neurons. Similarly the chemoreceptors of the chilaria and flabella of the arachnid, *Limulus polyphemus*, consist of an exoskeletal pore which is innervated by 6 to 15 sensory neurons (Barber and Hayes, 1963; Hayes, 1971; Wyse, 1971).

The structure within the pore has not been identified in the present study. If the pores are sensory receptors these structures may be nerve endings. Clear definition of their function must await a combined behavioural, electrophysiological and transmission EM study.

(b) Setal Morphology

Thomas (1970) has reported only plumose, acuminate and aesthetasc setae on the outer flagellum of the crayfish, *Austropotamobius pallipes*. In contrast, only smooth-walled setae were found on either antennular flagellum of *P. alaskensis*. Thomas (1970) further notes that all the setae of *A. pallipes* have a single annulation somewhere along their length. In *P. alaskensis* a single annulation was often seen on the dorsal and ventral setae, the type I and type II lateral-mesial setae, accessory setae and the setae of the most distal segment of the outer flagellum but never on the type I or type II setae of the inner flagellum.

The aesthetasc setae of *P. alaskensis* resemble those reported in the brachyurans, *Cancer productus*, *C. antennarius* and *Paragrapsus gaimardii*, the anomuran *Pagurus hirsutiusculus* and seven families of cryptoniscinid isopods (Ghiradella, Case & Cronshaw, 1968*b*; Cronshaw & Case, 1968, 1970; Nielsen & Strömberg, 1973; Snow, 1973) in that they have periodic annulations along much of their length. In contrast, only a single annulation is reported to occur in the aesthetascs of *A. pallipes* and none have been reported in the aesthetascs of several cyprid ostracods (Thomas, 1970; Danielopol, 1971).

Thomas (1970) considers that a general feature of all crustacean setae is an apical pore. In an SEM study of the setae of the crayfish, *A. pallipes*, he reports that indeed the setae of the crayfish have an apical pore which is 0·5 to 1·5 μm in diameter (Thomas, 1971). In the present study an apical pore of 0·4 to 1·0 μm has been found in all but the aesthetasc, type I lateral-mesial setae and the proximal dorsal setae of the outer flagellum. It must be stressed that the possible presence of secretions which might fill a pore mean that it is difficult to demonstrate the absence of even sizable pores using the SEM.

The question of the presence or absence of apical or other pores is of particular interest with respect to the chemoreceptive aesthetasc setae (Laverack, 1964; Laverack & Ardill, 1965; Ghiradella, Case & Cronshaw, 1968*a*,*b*; Ghiradella, Cronshaw & Case, 1968, 1970; Danielopol, 1971; Nielsen & Strömberg, 1973; Snow, 1973). Laverack (1968) has suggested that such pores would be important for the penetration of chemical stimulants to the sensory dendrites. Pores cannot, however, be considered a diagnostic characteristic of crustacean chemoreceptive setae for two reasons. Firstly, all setae in the crayfish, *A. pallipes*, have an apical pore (Thomas, 1971). In addition, on the antennules of *Pagurus alaskensis* many setae, for which a mechanoreceptive function will be argued below, have an apical pore. Secondly, not all crustaceans examined have been shown to have aesthetascs with an apical pore. Terminal pores have been reported in the aesthetascs of *A. pallipes*, *Panulirus argus*, the cryptoniscinid isopids *Cironiscus* and *Clypeoniscus* and several cyprid ostracods (Laverack & Ardill, 1965; Thomas, 1970, 1973; Danielopol, 1971; Nielsen & Strömberg, 1973). No pores, however, have been found in the distal portions of the aesthetascs of the decapods *C. productus*, *C. antennarius*, *Coenobita compressus*, *Panulirus interruptus*, *Pagurus hirsutiusculus* and *Paragrapsus gaimardii* or the cryptoniscinid isopod *Asconiscus* (Ghiradella, Case & Cronshaw, 1968*a*,*b*; Ghiradella, Cronshaw & Case, 1968, 1970; Nielsen & Strömberg, 1973; Snow, 1973). The absence of an apical pore suggests that a similar mechanism may also allow stimulant penetration into the aesthetascs of *Pagurus alaskensis*.

(c) Setal function

The antennules of *P. alaskensis* are not used for cleaning but appear to be highly sensitive to osmotic, mechanical and chemical stimuli (Section 2, Chapter III). All the setae of the antennular flagella are thus considered to have a sensory function.

Although morphological similarities may indicate similar sensory functions of setae, differences in setal morphology do not necessarily indicate differences in sensory function. Rather than setal morphology, more reliable determinants of setal function would seem to be: (1) their topographical location in relation to other surface structures and to the normal activities of the appendage or portion of the body on which they occur; (2) information from the limited behavioural, electrophysiological and transmission EM studies on crustacean setae. Unfortunately, detailed descriptions of setal arrangement and morphology are seldom given in electrophysiological and behavioural studies. Thus at present it is necessary to draw comparisons between the setae of different species on the basis of their general position.

The "companion" setae which Laverack (1964) describes as occurring near the aesthetascs of the lobster are most probably analogous to the accessory setae of the hermit crab antennule. The "companion" setae were found to be mechanoreceptors innervated by 2 to 4 sensory units. During a study of the antennular activities of *Pagurus alaskensis* I was able to elicit antennular wiping by mechanical stimulation of the aesthetascs (Section 2 *(c)*, Chapter III). It is possible that during these experiments, stimuli were actually being delivered to and registered by the accessory setae rather than the aesthetascs. Antennular wiping, however, is probably important in removing particles trapped amongst the aesthetascs and it is difficult to see how the accessory setae could register the presence of such particles. If the accessory setae are mechanoreceptors then their very close structural similarity to the type I setae of the inner flagellum might indicate that the latter are also utilized for transducing mechanical stimuli.

The type II setae of the more proximal segments of the inner flagellum could be considered to form a comb-like guard along the ventral approach to the aesthetascs while those on the distal segments form the most ventral extension of the resting antennule. Preliminary experiments have shown that mechanical stimulation of the proximal type II setae results in increased activity in antennular nerve 2. Pipetting water around the inner flagellum also resulted in prolonged activity in nerve 2, even after removal of the outer flagellum. Holmes and Homuth (1910), Copeland (1923) and Laverack (1964) have reported mechanical sensitivity of the inner flagellum of a variety of crustaceans. Chemical sensitivity of the inner flagellum has also been reported by Holmes and Homuth (1910) and Copeland (1923). The setae of the inner flagellum have an apical pore which may enable distilled water or chemicals to penetrate to the sensory neurons. Mechanical stimulation of the inner flagellum or pipetting distilled water around the antennular flagella of *P. alaskensis* was shown to be sufficient to elicit an extension-withdrawal reflex (Section 2 *(d)*, Chapter III) but both these types of stimuli could have affected both type I and type II setae. Extension-withdrawal reflexes are possibly important in removing the antennules from potentially noxious stimuli (see Section 3 *(d)*, Chapter IV). It is thus possible that the type I and type II setae function as polymodal sensilla that register potentially noxious stimuli. It is interesting to note that Bessou and Perl (1969) have described single polymodal sensory neurons in vertebrates which respond specifically to noxious stimuli.

The setae of the long, thin, distal segments of the outer flagella are most probably mechanoreceptors. Mechanical sensitivity of the outer flagella of a variety of crustaceans has been well documented (Holmes & Homuth, 1910; Copeland, 1923; Maynard & Dingle, 1963; Laverack, 1964). Furthermore, mechanical stimulation of these segments in *P. alaskensis* resulted in a fast flexion-withdrawal reflex but once again the stimuli were not specific enough to accurately localize the setae involved (Section 2 *(d)*, Chapter III).

Similarly, the proximal dorsal setae and the type I lateral-mesial setae are probably mechanoreceptors. Mechanical stimulation of the dorsal surface or sides of the outer flagella were sufficient to elicit a slow flexion-withdrawal reflex and such stimuli would almost certainly have affected these setae (Section 2 *(d)*, Chapter III). The extreme recumbence of these setae under the SEM and their absence on most of the fused short segments initially suggested that they may register bending of the outer flagellum across the short segments. In fresh material, however, these setae do not make contact with the more distal short segments, even on extreme distortion of the outer flagellum. Furthermore, Laverack's (1964) data suggest that the small setae near the joints between the short segments of the lobster outer flagella are mechanoreceptors while lateral bending of the outer flagella is registered by internal proprioceptors.

The setae of the antennular flagella must be subject to considerable stresses during antennular flicking in P. *alaskensis* (see Section 2 *(a)*, Chapter III). If, indeed, many of these setae are mechanoreceptors then one must imagine that each flick must elicit a mass of sensory discharge. Alternatively it is possible that the setae are either insensitive to deflections elicited by antennular flicking or that their sensory neurons are in a continual state of adaptation to such deflections.

The setal armature of the antennular flagella of P. *alaskensis* is sufficiently consistent to enable a detailed neurophysiological study of the receptive properties of specific types of setae which have a specified topographical location. It should also be possible to use partially restrained preparations (see Section 4 *(a)*, Chapter II) in combination with precisely localized stimuli and very specific setal abalations to gain much information regarding the whole-animal significance of the various setae. The ability of crustaceans to regenerate antennules then makes this a favourable system for looking at the re-establishment of specific receptor properties and central connections. Furthermore, because hermit crabs can now be successfully raised in the laboratory (Nyblade, 1970; Roberts, 1970) one may consider studying these areas in relation to the developmental process.

(2) Analysis of the Antennular Activities

(a) Antennular flicking

In seeking the major function of antennular flicking one is led to consider the most obvious result of this activity: the splaying of the aesthetasc setae. Although the movements during flicking are very rapid, they are also relatively small. Consequently it seems unlikely that they could generate water currents which are of significance to structures other than those on the antennules. In addition, the antennules are usually extended upwards in front of the crab so that antennular flicking is generally away from the body and other appendages. It could be suggested that flicking is important in removing debris caught amongst the aesthetasc setae but debris rapidly accumulates following the removal of the endopodites of the 3rd maxilliped and this operation does not inhibit flicking. I therefore propose that the function of antennular flicking in aquatic decapods is related to the function of the aesthetasc setae.

There is considerable behavioural, electrophysiological and ultrastructural evidence that the aesthetasc setae are of primary importance in distance chemoreception (Laverack, 1964; Laverack & Ardill, 1965; van Weel & Christofferson, 1966; Hazlett, 1968; Ghiradella *et al.* 1968*b*; Ache & Case, 1969; Snow, 1973). Marine brachyurans and anomurans have long (up to 1700 μm), thin (basal diameter: 10 to 30 μm) aesthetascs which are crowded into closely spaced rows on the outer antennular flagellum (Balass, 1944; Ghiradella *et al.* 1968*b*). In *Pagurus alaskensis* the maximum separation between the long rows of aesthetascs occurs near the bases of the setae and is only 80 μm. This suggests that there would be little exchange between the water surrounding the aesthetascs and that of the crab's immediate environment. The limitations which this structural organization may place on chemoreception are emphasized when one considers that probably only the distal portions of the aesthetascs of marine decapods are permeable to dissolved materials (Ghiradella *et al.* 1968*b*). Any mechanism which facilitates the circulation of water around the aesthetasc setae could thus be considered of primary importance to the chemoreceptive process.

In *P. alaskensis* there are two types of specializations which could be important to the circulation of water around the aesthetasc setae: (1) the specialized structure of the outer flagella and the aesthetasc setae; (2) the patterning of movements during antennular flicking.

1. *Structure of outer flagella and aesthetasc setae*

The aesthetascs of marine decapods may contain the processes of up to 400 sensory neurons per seta (Ghiradella *et al.* 1968*b*). Water resistance forces during antennular flicking result in bending of the aesthetascs and movement about their basal attachment with the outer flagellum. If the wall of an aesthetasc resembled a uniform cylinder, rigidly fixed to the surface of the outer flagellum, then these water resistance forces might be sufficient to cause distortion of the wall and damage to the densely packed sensory processes. The socket in which each aesthetasc of *P. alaskensis* is borne would allow some movement of each aesthetasc around its basal attachment while the periodic annulations along each aesthetasc may act as joints which permit bending of the setae without distortion of the cylindrical form (Plates 5 and 8a).

Flexibility of the basal attachment of the aesthetascs of *Cancer productus* and *P. hirsutiusculus* is suggested by figures 2 and 3 shown by Ghiradella *et al.* (1968*b*) and such structures have also been observed in *Paragrapsus gaimardii* (Snow, 1973) and in epicarid isopods (Nielsen & Strömberg, 1973). Structures which resemble the periodic annulations of the aesthetascs of *Pagurus alaskensis* have been observed in the aesthetascs of the brachyurans, *Paragrapsus gaimardii* (Snow, 1973), *Cancer antennarius* and *C. productus* (Ghiradella, Cronshaw & Case, 1970), in the anomuran, *Pagurus hirsutiusculus* (Ghiradella, Cronshaw & Case, 1968) and in seven families of cryptoniscinid isopods (Nielsen & Strömberg, 1973). Nielsen and Stromberg (1973), however, have suggested that these annulations may be important in retaining the cylindrical form in the resting aesthetasc.

In *P. alaskensis* there is extreme bending of the outer flagellum about 25 msec after the initiation of a flick. This results from water resistance forces acting on the long, thin distal segments of the outer flagellum to cause considerable bending across the distal shorter segments. The result of this bending is a more uniform separation between the aesthetascs of adjacent long rows. Furthermore, the medial part which divides the aesthetascs into a mesial and lateral group facilitates the lateral-mesial splaying of the aesthetascs. The outer flagella of many marine brachyurans and anomurans are of similar morphology to those of *P. alaskensis* (personal observation). In contrast, however, the outer flagellum of the semi-terrestrial hermit crab, *Coenobita compressus*, lacks the thin distal segments and the conical form of the outer flagellum of *P. alaskensis*. The aesthetascs of *Coenobita* are short pegs and (particularly in a gaseous medium) distortion of outer flagella (if possible) would not greatly increase contact of the aesthetascs with chemical stimulants.

2. *Patterning of movements*

During flicking in P. *alaskensis* the temporal relationship of tension in muscles which control flexion movements at the MS-DS and DS-OF joints appear to be important to the spreading of the aesthetasc setae. A flick is initiated by flexion at the MS-DS joint 2·5 msec before movement of the DS-OF joint. This infers that tension development in muscle 32F which produces fast flexion at the DS-OF joint occurs only after the development of tension in muscle 31F which produces fast flexion at the MS-DS joint. Myogram recordings from these muscles in fact show that electrical activity in muscle 31F precedes activity in muscle 32F by 2·3 to 4·2 msec. Flexion at the MS-DS joint is interrupted for about 5 msec following flexion at the DS-OF joint, but this interruption is not observed following excision of all but the basal segment of the outer flagellum. Furthermore, there is no consistent interruption of the electrical activity in muscle 31F which could account for the interruption in flexion at the MS-DS joint (Section 4 *(b)*, Chapter III). It is thus possible that the contraction of muscle 31F before muscle 32F may be important in preventing extension at the MS-DS joint resulting from water resistance forces generated by flexion at the DS-OF joint. Such a mechanism would ensure that the outer flagellum is moved through sufficient water to spread the aesthetasc setae.

Unfortunately the timing of movements within a single flick have not been precisely described in other crustaceans. Preliminary observations suggest that the morphology of the antennules of most aquatic decapods either resembles that described in *P. alaskensis* or that described in lobsters and shrimps (Maynard & Dingle, 1963; Ache & Case, 1969). The outer flagellum of the latter type of antennule is long and thin and bears little resemblance to the short, conical outer flagella of *P. alaskensis*. Decapods with antennules similar to those of *P. alaskensis* show relatively high frequency of vigorous flicks but, in contrast, in species with antennules resembling those of shrimps and lobsters antennule flicks occur less frequently and are less vigorous (personal observation). Maynard and Dingle (1963) report that single flicks of the lobster antennule may involve movement of the outer flagellum only. More frequently, however, they observed a series of flicks of the outer flagellum which were accompanied by a tonic flexion of the MS-DS joint which lasted for the duration of the series. In lobsters the rows of aesthetascs are borne on the distal portion of a long (about 20 cm), flexible outer flagellum and are separated by approximately 250 μm (Laverack, 1964). A series of flicks of the outer flagellum might thus be sufficient to exchange the water around the aesthetascs despite the length and flexibility of the outer flagellum.

A comparative study of the structure and activity of the antennules of various aquatic decapods may reveal a strong correlation between the vigour and frequency of antennular flicking, the density of the aesthetasc setae and the size and morphology of the outer flagellum. Experimentally, however, the most conclusive test of whether flicking is important to chemoreception may come from attempts to record gross activity from the aesthetasc sensory nerve (nerve 1)

(Section 3 *(a)*, Chapter III) during antennular flicking in a chemically changing environment.

If flicking is essential for the circulation of water around the aesthetasc setae then it could be considered that a crab only senses changes in dissolved chemicals upon flicking an antennule. Although the inter-flick interval is highly variable in a constant environment, the mean inter-flick interval may be decreased by increasing the water currents or fish odour in the crab's surroundings. In addition, certain stimuli result in an interruption of flicking. These observations suggest that the inter-flick interval may be determined by the selective advantage of flicking, firstly in relation to recent sensory conditions and secondly under the immediate sensory conditions. Assuming that flicking does provide a means of phasically sampling the dissolved chemicals in a crab's immediate environment, the plasticity of the inter-flick interval might thus provide a means of directing attention towards or away from chemical stimuli. It is difficult, however, to see why a central neuronal circuit would not be a more economical means of achieving the same ends.

(b) Antennular rotation

In the lobster the antennules are frequently pointed towards the source of chemical stimuli. These pointing movements frequently form part of more complex 'exploring-feeding' sequences (Maynard & Dingle, 1963). Similarly, antennular rotation in the hermit crab, *P. alaskensis*, was most frequent when crabs appeared to be exploring new surroundings or when there was a high mean frequency of flicking.

In the aquatic environment the movement of chemical stimulants would be heavily dependent on water currents. In general the antennules are considered to be important to distance chemoreception (Hazlett, 1968, 1971*b*). Furthermore, it has been argued above that flicking facilitates the circulation of water around the aesthetasc setae which are probably the major sites of antennular chemoreception (Laverack, 1964; Ghiradella *et al.* 1968*b*). The splaying of the aesthetasc setae would be maximal when flicking is directed into existing water currents. Antennular rotation might thus be important to chemoreception by ensuring that flicking is usually against water currents. The antennules are often orientated so that the aesthetascs point into existing water currents. It would thus be interesting to attempt to find what receptors are involved in this response (see Brock, 1930; Luther, 1930; Laverack, 1962). A second important question is whether the rotation of the antennules is important to orientation of the whole animal towards a food source in the presence and absence of water currents (see Brock, 1926; Hazlett, 1968; Burrows & Willows, 1969; Charlton, 1971).

(c) Antennular wiping

The function of wiping is probably to remove debris caught amongst the aesthetasc setae. In high densities such materials would greatly impede the exchange of water around the aesthetascs which is suggested above to be of importance to the chemoreceptive process. During wiping in *Pagurus alaskensis* the long comb-like setae on the endopodites of the 3rd maxillipeds probably pass through the rows of aesthetasc setae removing any trapped material. In contrast, in the sand crab *Emerita*, the antennules are cleaned with a group of setae on the fifth antennal segment (Efford, 1971). The flexibility of the aesthetascs around their basal joints may be important in preventing damage from the endopodites or the antennae.

In attempting to determine the function of an activity it is important to consider the modality of the triggering stimulus. In the hermit crab wiping may be elicited by light mechanical stimulation of the aesthetasc setae, but pure chemical stimuli were ineffective. Efford (1971) has noted that the sand crab *Emerita* wipes its antennules when graphite particles become caught amongst the aesthetascs. In contrast, Maynard and Dingle (1963) report that wiping in the lobster was best elicited by chemical or chemotactile stimuli and only occasionally followed mechanical stimulation. Stimulation of mechanoreceptors situated near the aesthetasc setae may be sufficient to elicit wiping in the hermit crab, but in lobsters the increased responsiveness to chemotactile stimuli means that specific chemotactile sensilla cannot be ruled out (see Laverack, 1964; Hazlett, 1971*a*). The observations that wiping in the sand crab, hermit crab and lobster occur in the absence of apparent stimulation and that in the lobster chemical stimulation alone appears to be a sufficient stimulus, possibly means that only very small particles are necessary to elicit this activity. Any facilitation of wiping by chemical stimuli would be selectively advantageous, as soluble materials trapped among the aesthetasc setae could result in an invalid interpretation of the chemical environment.

(d) Antennular withdrawal

Although no attempt has been made to elicit antennular withdrawal by stimulating specific receptors, both mechanical and osmotic stimulation of the antennules have been used successfully. The electrophysiological experiments of Krijgsman and Krijgsman (1954), Laverack (1964) and van Weel and Christofferson (1966) have demonstrated the presence of mechanoreceptors and osmotically sensitive receptors on the antennules of various decapods, and the evidence of Maynard and Cohen (1965) has suggested that some fast-conducting antennular mechanorectors have a monosynaptic, excitatory influence on an antennular motoneuron in the lobster.

In both the lobster (Maynard & Dingle, 1963) and the hermit crab an extension reflex could be elicited by mechanical stimulation of the inner flagellum while a flexion reflex resulted from stimulation of the outer flagellum. Both the extension and flexion reflexes thus move the antennule away from the stimulus. Similarly, prolonged osmotic stimulation of the antennules in the hermit crab sometimes resulted in flexion and then extension of the antennule in what appears to be an attempt to remove the antennule from the stimulus. Mechanical or osmotic stimulation could be interpreted as being potentially noxious to the aesthetasc setae and other antennular structures and thus antennular withdrawal may be regarded as a purely protective response.

The form of extension withdrawal in the lobster is similar to that described in the hermit crab and involved extension at the MS-DS joint (Maynard & Dingle, 1963). In the hermit crab both fast and slow flexion-withdrawal reflexes are recognizable. A slow withdrawal reflex involves a smooth, slow flexion at both the MS-DS and DS-OF joints while a fast withdrawal reflex involves a rapid flexion at only the MS-DS joint. Although flexion-withdrawal reflexes in the lobster usually involve flexion at both the MS-DS and DS-OF joints it is not clear that what have been defined as fast and slow flexion-withdrawal reflexes in the hermit crab are not both involved in flexion withdrawal as described in the lobster (see Maynard & Dingle, 1963).

In the hermit crab, fast flexion-withdrawal reflexes could be elicited by strong taps to the eyestalks or shell or by bending the tip of the outer flagellum towards the aesthetasc setae. In contrast, however, slow flexion-withdrawal reflexes could be elicited by touching the eyestalks, antenna, carapace or sides or dorsal surface of the outer flagellum. These observations suggest that the fast flexion-withdrawal reflex serves to rapidly remove the antennules from a stimulus which may cause immediate damage to the antennules while the slow flexion-withdrawal reflex serves to temporarily adjust the posture of the antennules so as to avoid repetition of a less severe stimulus.

In the hermit crab repeated mechanical stimulation of the antennules, eyestalks, antennae, legs or body, or continual stimulation of the antennules with distilled water, results in tonic flexion of both antennules as well as in postural modifications of the eyestalks and antennae. This response appears to be an avoidance which provides maximum protection of the antennules from the possibly noxious effects of these stimuli. In the natural state this tonic withdrawal posture would provide protection of the antennules in animals occupying shells too small to permit their complete withdrawal or in animals disturbed in the process of changing shells.

It is interesting to note that many brachyurans withdraw their antennules by folding them into a groove in the ventral side of the cephalic sternum (personal observation). This folding involves movement at the MS-DS joint which is in the opposite direction to flexion at this joint in the hermit crab antennule. Although such differences may be purely the result of differences in the anatomy of the antennular musculature (cf. Schmidt, 1915; Cochran, 1935; Section 3 *(a)*, Chapter III), a comparative behavioural and neuromuscular study of the brachyuran and macruran antennules may provide interesting examples of the adaptations of a neuromuscular system.

(3) Motor Innervation and Musculature

(a) Organisation of the musculature

No overshooting EJPs were observed in any muscles studied. The fibres of these muscles could, however, be divided into two groups on the basis of the rates of decay of their EJPs, their sarcomere length and their rates of contraction and relaxation. Atwood (1965) has shown that the EJPs in the rapidly relaxing, short-sarcomered, thick fibres of the opener muscle of *Chionectes* have a shorter time constant than those in the slowly relaxing, long-sarcomered, thin fibres. Although recording of tension from single muscle fibres of the antennular muscles was not practicable, the present experiments show that EJPs with slow rates of decay occurred in muscle fibres with long sarcomeres and were always threshold-linked with slow rates of contraction and relaxation. In addition, EJPs with fast rates of decay were recorded in muscles which had short sarcomeres and were threshold-linked with fast rates of contraction and relaxation.

A possible exception to this scheme are those fibres of muscle 30 which are innervated by motoneuron A30F. The EJPs of motoneuron A30F decayed rapidly and could be threshold-linked with a brief twitch in muscle 30, yet fibres with short sarcomeres were only rarely observed in this muscle. Frequently fibres innervated by motoneuron A30F were difficult to find, suggesting that they represent only a small percentage of the fibre population of muscle 30. During the measurements of sarcomeres a few fibres with sarcomere lengths of about 3·5 µm were found in three preparations of muscle 30. Failure to discover such fibres in other preparations may only reflect their scarcity.

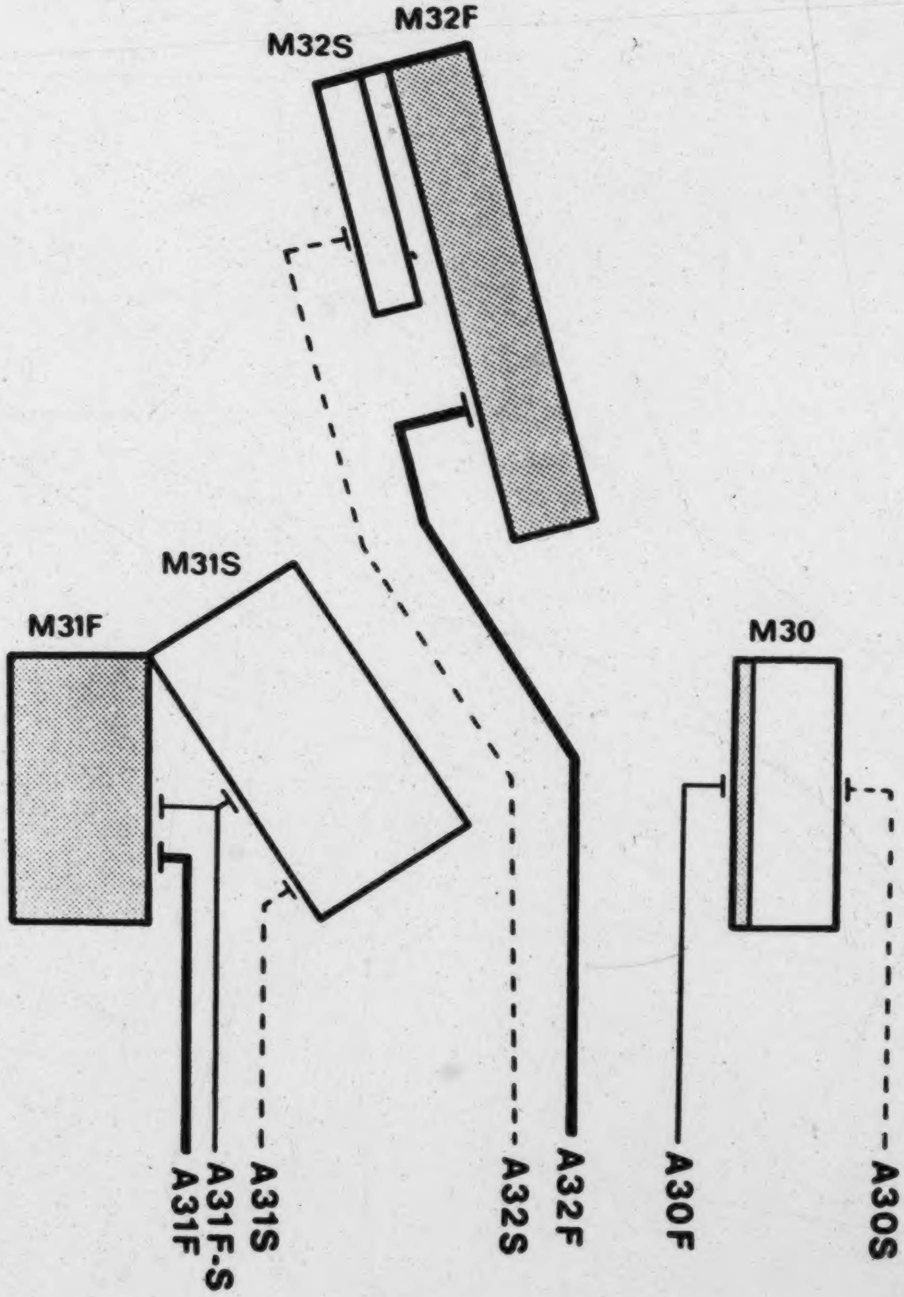
(b) Comparison with other systems

The neuromuscular organization of the antennular motor system is summarized in Figure 27. The motor system can be divided into a phasic component, a tonic component and a phaso-tonic component. The phasic component consists of motor units 30F, 31F and 32F, muscles 31F and 32F and the fast fibres in muscle 30. The tonic component consists of motor units 30S, 31S and 32S (motoneurons A30S, A31S and A32S, muscles 31S and 32S and the slow fibres of muscle 30). Finally, the phaso-tonic component consists of a single motor unit 31F-S (motoneuron A31F-S and muscles 31F and 31S).

With the exception of motoneuron A31F-S there is no sharing of motoneurons between anatomically separate muscles. This situation

Fig. 27. Schematic diagram of the motor innervation and musculature of the distal and medial antennular segments. Stippled areas of the blocks represent fast muscle cells while unmarked areas represent slow muscle cells. Continuous lines represent motor axons whose EJPs do not facilitate. The thicker lines represent the giant motor axons A31F and A32F. Broken lines represent motor axons whose EJPs show facilitation.

(27)


M32S
M32F
M31S
M31F
M30
A31F
A31F-S
A31S
A32S
A32F
A30F
A30S

parallels that of the crayfish uropods (Larimer & Kennedy, 1969) and, with the exception of the common excitor to the stretcher and opener muscles, that of the crustacean leg (Wiersma & Ripley, 1952; Sherman & Atwood, 1971). The anatomical distribution of motoneuron A31F-S differs from that of the common excitor of the crustacean leg in that it innervates synergistic muscles (M31F and M31S) and in this respect it is more analogous to motoneuron D_s which innervates four of the six cockroach mesothoracic and metathoracic coxal depressor muscles (Pearson & Iles, 1971). Separate synergistic bundles with shared motoneurons have been identified in the lobster swimmeret system, but Davis (1968*a*) has chosen to consider these as parts of a single functional muscle whose division simply represents an optimal mechanical arrangement of the muscle and its attachments within an irregular enclosure.

A second interesting feature of the antennular system is that, with the exception of motoneuron A31F-S, all motoneurons innervate either fast or slow muscle fibres (Fig. 27). A rigid separation between the innervation of fast or slow muscle fibres has been documented in the crayfish abdominal musculature (Kennedy & Takeda, 1965*a*,*b*; Parnas & Atwood, 1966). In contrast, however, the motoneurons of the crustacean leg innervate a mixed population of muscle fibres (see Hoyle & Wiersma, 1958; Atwood, 1963). The crayfish abdomen and the crustacean leg represent extremes, and more intermediate situations are found in the crayfish uropods and the cockroach leg. Most motoneurons to the uropod muscles innervate only fast or slow fibres but in at least one case Larimer and Kennedy (1969) observed a motoneuron innervating both fast and slow fibres of a mixed muscle. Similarly, although the four fast coxal depressor muscles of the cockroach leg are innervated by a single motoneuron D_f, the two slow muscles and two of the fast muscles are innervated by a single motoneuron D_s (Pearson & Iles, 1971; Iles & Pearson, 1971). In both these systems the motoneurons innervating both fast and slow muscle fibres may be compared with motoneuron A31F-S in the antennular motor system.

An interesting feature of the slow motor units 30S, 31S and 32S is the very small, slow tension responses which may be elicited by a single stimulus to the motor nerve. Such responses facilitate the development of extremely small tonic tensions upon low-frequency (5 to 10/sec) excitation of the slow motoneurons (Figs. 9AIII, 12B and 15A,B). During extension-withdrawal and slow flexion-withdrawal reflexes, posturing of the antennule at the MS-DS joint and tonic flexion withdrawal, the frequency of spikes in motoneurons A30S, A31S and A32S is sometimes only 15/sec. Thus if the motoneuronal frequency - muscle tension relationships of the slow motor units are similar to those described in excised antennules, then this phenomena could be regarded as extending the low-frequency response range of the slow muscle fibres to motoneuronal activity. Functionally this would be extremely useful for the fine postural control of appendages which, like the antennules, have little joint resistance and whose weight is largely balanced by the density of the environmental medium. A similar mechanism, however, has been shown to contribute to the net tension produced in the crayfish slow abdominal flexor muscles by bursts in the largest motoneuron (Gillary & Kennedy, 1969).

(4) Patterns of Activity in the Antennular Motoneurons

(a) Functional significance of motoneuronal activity during antennular flicking

In *Pagurus alaskensis* an antennular flick consists of a flexion and then an extension at the MS-DS and DS-OF joints. The flexion phase of a flick results from a burst of spikes in motoneurons A31F and A32F. At the level of the proximal segment the first spike in motoneuron A31F is considered to precede the first spike in motoneuron A32F by 1·8 to 2·1 msec. The delay between the bursts in these motoneurons thus results in the first EJP in muscle 31F preceding the first EJP in muscle 32F by 2·3 to 4·2 msec. This sequence of activity is considered to be functionally important in preventing extension at the MS-DS joint during flexion at the DS-OF joint and thus in ensuring splaying of the aesthetasc setae (Section 2 *(a)*, Chapter IV).

From 20 to 40 msec after the first spike in flexor motoneuron A31F a burst of usually 1 or 2 spikes occurs in extensor motoneuron A30F. This would be expected to result in a twitch of the fast fibres of muscle 30 and rapid extension at the MS-DS joint. During a flick, extension at the MS-DS joint begins 28 to 37 msec after the initiation of flexion at this joint (Table 2) suggesting that the extension phase of most flicks results solely from activity in motoneuron A30F. Some flicks were not accompanied by activity in motoneuron A30F even though the antennule was in an extended position. In these cases it is necessary to attribute extension to MS-DS joint elasticity. During all movements, extension at DS-OF joint is considered to be purely the result of joint elasticity.

The length and number of spikes in the flexor bursts is highly variable in many animals. It has been argued above that the sequence of activity in flexor motoneurons A31F and A32F is adapted to produce a strong movement of the outer flagellum through the water, thus ensuring the exchange of water around the chemoreceptive aesthetasc setae. An extension of this hypothesis is that longer flexor bursts result in an increase in the amplitude and duration of flexion at the MS-DS and DS-OF joints and thus a more complete exchange of water around the aesthetasc setae. This could result in a purer sample of the water in the crab's immediate surroundings and so a more distinct sensing of any changes in dissolved chemicals.

Visual observations have suggested that longer flexor bursts do result in a greater degree of flexion at the MS-DS joint. This would be expected as during a flick the flexor motoneurons fire with frequencies in the range of 170 to 270/sec. A single stimulus to motoneuron A31F or A32F results in a twitch contraction with a total duration of 20 to 25 msec in muscle 31F or 32F, respectively. Even considering the longer inter-spike intervals during flexor bursts, it seems likely that summation of single twitches would occur on the firing of motoneurons A31F and A32F at their "natural" frequencies. For example, the occurrence of 2 or 3 spikes in a single motoneuron of the fast, dorsal, longitudinal flight muscle of the locust, with an inter-spike interval of 4 to 8 msec, produces a smooth twitch of 50% more tension than is elicited by a single spike (Wilson & Weis-Fogh, 1962; Neville & Weis-Fogh, 1963). It should, however, be strongly emphasized that the relationship between motoneuronal discharge patterns and muscle contractions may be complex (see: Spirito, Evoy & Fourtner, 1973).

There is some evidence to suggest that the longest flexor bursts may be of no functional significance but rather might represent an unnecessary feature of the underlying burst-generating mechanism. This proposal hinges on the observation that very long flexor bursts often result in an overlap between electrical activity in flexor motoneuron A31F and extensor motoneuron A30F. The duration of tension in locust flight muscle is directly determined by the duration between the first and the last closely spaced motoneuronal spikes (Neville & Weis-Fogh, 1963). If a similar tension-burst length relationship exists in flexor motor unit 31F then it is almost certain that tension would be developed synchronously in the fast portion of extensor muscle 30 and in flexor muscle 31F. The difficulty in suggesting a function for synchronous tension development in antagonistic fast muscles leads one to conclude that the longest bursts in motoneuron A31F may be without functional significance.

(b) Neuronal control of antennular withdrawal

During a fast flexion-withdrawal reflex the MS-DS joint is flexed rapidly through 50 to 80°. This reflex is always accompanied by a burst of EJPs in flexor muscles 31F and 31S resulting, presumably, from a burst of spikes in motoneuron A31F-S. The proximal attachment of muscle 31S is such that any tension developed would be most effective following partial flexion while tension in muscle 31F would become less effective as flexion continued (Fig. 1). A single action potential in motoneuron A31F-S could therefore elicit rapid but brief flexion at the MS-DS joint via muscle 31F which would then be maintained, or even augmented, by tension in muscle 31S. A relatively low-frequency (10/sec) burst in motoneuron A31F-S could thus easily overcome water resistance forces which would oppose the large and rapid flexion movement constituting the fast flexion-withdrawal reflex (Figs. 7A and 10A,B).

There is evidence that during antennular flicking contraction of muscle 31F and rapid flexion at the MS-DS joint might be sufficient for excitation of extensor motoneuron A30F (Section 5 *(b)*, Chapter III). Therefore one feature of the fast flexion-withdrawal reflex which must be accounted for is that the contraction of muscle 31F and rapid flexion at the MS-DS joint never result in excitation of motoneuron A30F. One must suppose that the stimuli which excite motoneuron A31F-S also result in inhibition of extensor motoneuron A30F. Rapid protective reflexes might thus be considered to fit with the general concept of escape behaviour, that at the threshold level of excitation the escape response (or fast withdrawal reflex) is elicited and all other activities and postures are terminated (Roberts, 1968).

Slow flexion-withdrawal reflexes are the result of activity in the slow flexor motoneurons A31S and A32S, while extension-withdrawal reflexes are the result of activity in slow extensor motoneuron A30S. Sometimes there was an overlap of activity in motoneurons A31S and A32S with activity in motoneuron A31F-S. Whether the resultant movements are classified as a fast or a slow flexion-withdrawal reflex probably depends on the relative burst lengths and intra-burst frequencies in motoneurons A31F-S and A32S (see Section 2 *(d)*, Chapter III). It seems best to consider that flexion-withdrawal reflexes are graded in intensity from those which result from a train of spikes in motoneurons A31S and A32S just sufficient to cause flexion at the MS-DS and DS-OF joints, to those which result solely from a high-frequency burst of spikes in motoneuron A31F-S causing the powerful flexion at the MS-DS joint characteristic of the fast flexion-withdrawal reflex (Section 2 *(d)*, Chapter III).

The most striking aspect of the motoneuronal activity during extension-withdrawal and slow flexion-withdrawal reflexes is the reciprocity which exists between high-frequency activity in flexor motoneurons A31S and A32S and extensor motoneuron A30S. Evidence is presented to suggest that sensory input which results in excitation of flexor motoneurons A31S and A32S also inhibits activity in extensor motoneuron A30S. Similarly, sensory inputs which excite motoneuron A30S probably also inhibit motoneurons A31S and A32S. It is not known whether this interaction between the afferent and efferent neurons is direct or, mediated via one or more layers of interneurons. Stimulation of the inner flagellum elicits extension withdrawal and inhibition of flexor activity in the lobster antennule (Maynard & Dingle, 1963; Maynard, 1965). Furthermore, Maynard and Cohen (1965) suggest that many receptors on the antennular flagella influence the antennular motoneurons which are involved in withdrawal reflexes via at least one layer of interneurons.

In the hermit crab repeated mechanical stimulation of the antennules, eyestalks, antennae, legs or body, or continual stimulation of the antennules with distilled water results in tonic flexion of both antennules at the MS-DS and DS-OF joints and postural modifications of the eyestalks and antennae. This posture has been classified as tonic flexion withdrawal and it is always associated with a train of spikes in motoneurons A31S and A32S. The DS-OF joint is extended first, while continued activity in motoneuron A31S maintains flexion at the MS-DS joint even after antennular flicking has been resumed.

Sometimes low-frequency activity (mean: 7·4/sec) was recorded simultaneously in antagonistic motoneurons A30S and A31S. It is possible that this activity is important in maintaining slight tension in muscles 31S and 30 which could stabilize the MS-DS joint against passive flexions or extensions induced by fluctuations in water currents. This would allow more controlled positioning of the antennules in the crab's surroundings.

Within the postural control system of the crayfish abdomen, Kennedy, Evoy, Dane and Hanawalt (1967) have shown that activity in specific command interneurons initiates and maintains specific abdominal postures. Furthermore, the activity in single motoneurons may be controlled by the frequency of spikes in a single command element (Evoy & Kennedy, 1967). Within the tonic component of the antennular motor system (motor units: 30S, 31S and 32S) of the hermit crab four discrete output patterns may be recognized. Thus one might propose that the tonic motor units are controlled by as few as four interneuronal command elements.

Threshold excitation of one command element could result in an extension-withdrawal reflex by exciting motoneuron A30S while inhibiting motoneurons A31S and A32S. Another command element could result in tonic flexion at the MS-DS joint by exciting motoneuron A31S and inhibiting motoneuron A30S. Another could result in tonic low-frequency activity in motoneurons A31S and A30S which may play a role in stabilizing the MS-DS joint to water current fluctuations. Finally, still another could result in excitation of both motoneuron A31S and motoneuron A32S but inhibition of motoneuron A30S. The duration of activity in this last command element could determine whether tonic flexion withdrawal follows a slow flexion-withdrawal reflex. In all cases the level of activity in the command interneurons could control the level of activity in the relevant motoneurons and thus be used to grade the velocity of the movements or the amount of postural change.

(5) Central Patterning and Reflex Control of Antennular Flicking

(a) Flexor activity

A flick is always initiated by a burst of spikes in both fast flexor motoneurons. The dependence of the flexor patterns on central patterning versus sensory input will be considered in relation to three major questions: (1) How is activity in the flexor motoneurons initiated? (2) How is the number of spikes/burst determined? (3) How is the timing between bursts in motoneuron A31F and bursts in motoneuron A32F determined?

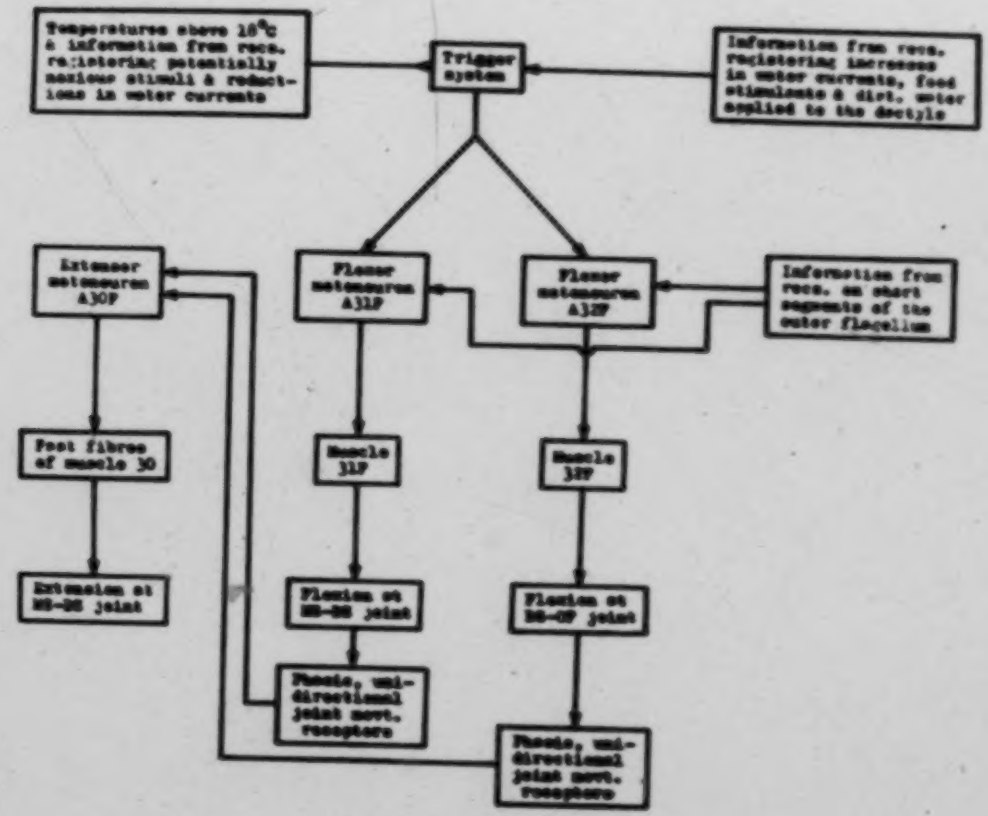
As yet specific stimuli have been unsuccessful in eliciting single flicks. Furthermore, there is no clear coordination of flicking between left and right antennules. Although flicks of either antennule occur at irregular intervals (i.e. non-rhythmically) some sensory parameters such as the presence or absence of water currents can alter the mean flicking frequency (Section 2 *(a)*, Chapter III). Neither these nor any other stimuli appear to influence the number of spikes/burst or the timing between bursts in the flexor motoneurons. Thus the flexion movements at the MS-DS and the DS-OF joints during a flick fulfil the major requirement of a triggered movement—that "the intensity of the initiating stimulus must reach 'threshold' but the movement itself will not vary as a function of suprathreshold variations in stimulus intensity" (Bizzi & Evarts, 1971).

In addition to suggesting that the flexion phase of a flick is triggered, I propose that: (1) activity in both flexor motoneurons of a single antennule results from activation of a single trigger system and (2) that the flexor activity in each antennule is the result of activation of a separate trigger system (Fig. 28). This trigger system may be a single cell, a group of cells or even part of one or both of the flexor motoneurons. Information from receptors which register increases in water currents, distilled water applied to the dactylopodites and, in some animals, or possibly some physiological states, the introduction of food stimulants, are considered as having a strong excitatory effect on the trigger system (Fig. 28). Furthermore, increasing the temperature above 18°C and information from receptors registering potentially noxious stimuli or reductions in water currents are considered as having an inhibitory effect on the trigger system (Fig. 28) (Section 2 *(a)*, Chapter III).

Bursts of more than 1 or 2 spikes in flexor motoneurons A31F and A32F are rarely seen following excision of the short segments of the outer flagellum. This is true even when a series of flicks occurs with a short mean inter-flick interval. It thus seems simplest to

Fig. 28. Model of the neuronal mechanisms underlying antennular flicking of a single antennule. Arrows represent excitatory inputs. The reversed arrow represents inhibitory inputs to the trigger system. Information from receptors (recs.) sensitive to increases in water currents, distilled water applied to the dactylopodites and in some animals, food stimulants, have a strong excitatory effect on the trigger system. Temperatures above 18°C and information from receptors registering reductions in water currents and potentially noxious stimuli have an inhibitory effect on the trigger system. Activation of the trigger system results in activation of flexor motoneurons A31F and A32F. Information from receptors on the short segments of the outer flagellum is necessary for flexor bursts of more than 2 spikes. The effectiveness of this information is regulated by the physiological state of the animal. Activation of extensor motoneuron A30F is dependent on feedback from phasic, unidirectional, joint-movement receptors at both the MS-DS joint and the DS-OF joint. This feedback reflexively excites extensor motoneuron A30F. See text for further details.

(2B)


Temperatures above 18°C & information from recs. registering potentially noxious stimuli & reductions in water currents
Trigger system
Information from recs. registering increases in water currents, food stimulants & dirt. water applied to the dactyls
Extensor motoneuron A30F
Flexor motoneuron A31F
Flexor motoneuron A32F
Information from recs. on short segments of the outer flagellum
Fast fibres of muscle 30
Muscle 31F
Muscle 32F
Extension at MS-DS joint
Flexion at MS-DS joint
Flexion at DS-OF joint
Phasic, uni-directional joint movt. receptors
Phasic, uni-directional joint movt. receptors

propose that flexor bursts of more than 1 or 2 spikes are the result of information originating from the many thousands of receptors on the short segments of the outer flagellum (Ghiradella, Case & Cronshaw, 1968*b*; Snow, 1973; Section 1 *(b)*, Chapter III). This information could influence the number of spikes/burst by increasing the excitability of both flexor motoneurons to activity in the trigger system (Fig. 28). The low proportion of bursts of more than 2 spikes in some intact animals further suggests that certain physiological states might result in the suppression of a large number of flexor spikes despite the presence of the receptors on the short segments of the outer flagellum.

At the level of the proximal segment the first spike in motoneuron A31F precedes the first spike in motoneuron A32F by 1·8 to 2·1 msec. Within any animal this lag is quite constant and is not changed by any alteration of sensory input. It is possible that this delay results from motoneuron A32F having a higher threshold than motoneuron A31F. Activation of the trigger system would thus cause depolarization of both motoneurons but earlier spiking of motoneuron A31F (Fig. 28). This mechanism could also account for the fact that the mean intra-burst frequency of motoneuron A32F is usually less than the mean intra-burst frequency of motoneuron A31F. One problem with this hypothesis is that even in preparations where the mean intra-burst frequency of motoneuron A32F is greater than that of motoneuron A31F, there is still a delay following the first spike in motoneuron A31F before the first spike in motoneuron A32F. Alternative hypotheses such as monosynaptic excitation of motoneuron A32F by a collateral of motoneuron A31F are possible. Furthermore, there is no necessity to attribute the timing of the flexor bursts and the intra-burst frequencies to the same factor or factors. The present model must be regarded as tentative (Fig. 28). Its major advantage is that it is simple and should be relatively easy to test by recording and stimulating intracellularly in the central processes of the flexor motoneurons.

(b) Extensor activity

Following the flexion phase of a flick extension at the MS-DS joint results from activation of the fast fibres of extensor muscle 30 by a burst of 1 to 4 spikes in motoneuron A30F. In contrast, extension at the DS-OF joint is the result of joint elasticity, and its rate and duration are probably determined by water resistance forces acting on the outer flagellum (Section 2 *(a)*, Chapter III). The present observations suggest that the activation of extensor motoneuron A30F is usually dependent on the movements at the MS-DS and DS-OF joints during the flexion phase of a flick. The prevention of flexion at the MS-DS joint usually results in a reduction of both the proportion of flicks correlated with extensor activity and the proportion correlated with more than a single extensor spike. Prevention of flexion at both the MS-DS and DS-OF joints further reduces the proportions of both these parameters, sometimes resulting in the complete abolition of flicks showing extensor activity (Table 10). This progressive abolition of extensor activity with progressive abolition of joint flexion suggests that activation of extensor motoneuron A30F might have one of the basic properties of a simple reflex. That is that the magnitude of the reflex movement varies with the magnitude of the input (Bizzi & Evarts, 1971).

It is useful to consider whether sufficient time is available for reflex activation of extensor motoneuron A30F. During flicking the first spike in motoneuron A30F usually occurs about 30 msec after the first spike in flexor motoneuron A31F (Table 5). High-intensity stimulation of muscle 31F elicits an EJP of motoneuron A30F after a delay of only 10 to 14 msec (Fig. 26). Observations suggest that this results from direct stimulation of sensory neurons which in turn excite extensor motoneuron A30F. If this is true then there remains only 16 to 20 msec for excitation-contraction coupling in flexor muscle 31F, joint movement and excitation of the receptors which excite motoneuron A30F. In fact, recordings from nerves 2 and 3 show that threshold stimulation of muscle 31F does result in a burst of spikes the first of which follows the stimulus artifact by only 10 to 15 msec. It is thus entirely possible that during flicking extensor motoneuron A30F is excited by sensory feedback resulting from the activity in flexor motoneurons A31F and A32F.

It seems likely that activation of phasic, unidirectional, movement receptors sensitive to joint flexion is necessary for the activation of extensor motoneuron A30F (Fig. 28). Firstly, phasic, unidirectional, movement receptors have been reported in the chordotonal organs of the MS-DS and DS-OF joints of the lobster antennule (Wyse & Maynard, 1963, 1965). Secondly, these receptors would theoretically be inactivated by immobilizing the antennule joints in a partially extended position. In contrast, this manipulation would not abolish feedback from any receptors responding to muscle tension (Macmillian & Dando, 1972) and does not influence activity in flexor motoneurons A31F and A32F. Thirdly, in crustacean chordotonal organs phasic, unidirectional, movement receptors are considered as having the largest axons and thus the highest conduction velocities (Burke, 1954; Wyse & Maynard, 1965; Hartman & Austin, 1972). In a few animals the first spike in extensor motoneuron A30F occurs only 20 msec after the first spike in flexor motoneuron A31F (Table 5). A high afferent conduction velocity would thus be advantageous for the reflex excitation of motoneuron A30F (see Burke, 1954).

Threshold stimulation of flexor muscle 31F was only occasionally sufficient stimulus to excite extensor motoneuron A30F. This stimulus results in antidromic activation of motoneuron A31F and tension development in muscle 31F as well as flexion at the MS-DS joint. As during a flick, flexion at the MS-DS joint is necessary for the excitation of extensor motoneuron A30F. It has not, however, been shown that flexion at the MS-DS joint is sufficient for excitation of extensor motoneuron A30F in the absence of antidromic activation of motoneuron A31F and tension in muscle 31F. Thus it is not known whether antidromic activation of motoneuron A31F or tension in flexor muscle 31F are also necessary for the excitation of extensor motoneuron A30F.

There are two factors that might account for the failure of all stimuli to muscle 31F to excite extensor motoneuron A30F. Firstly, stimulating muscle 31F results in flexion at the MS-DS joint but not the DS-OF joint. During flicking, flexion at both joints seems to contribute to the excitation of motoneuron A30F. Secondly, it may be that during a flick there is some centrally patterned, subthreshold excitation of extensor motoneuron A30F. This could facilitate excitation of motoneuron A30F in response to sensory feedback.

In a few animals, apparently complete abolition of joint movement resulted in the abolition of extensor activity in only 60% of flicks. It has been argued above that extensor motoneuron A30F is excited by feedback from phasic, unidirectional, joint-movement receptors. Macmillian and Dando (1972) emphasize the extreme difficulty in abolishing feedback from crustacean chordotonal organs by joint immobilization. In the present experiments a joint was considered immobilized when it could not be seen to move during periods of electrical activity in the related muscles. It is thus completely possible that in some preparations small and undetected joint movements were occasionally sufficient to excite receptors capable of activating extensor motoneuron A30F.

(c) The functional significance of reflex control

To date there is no evidence for excitation of any component movement of a stereotyped activity being completely dependent on activation of receptors by preceding component movements. Proprioceptive input has been shown to influence phasic motor systems in two major ways. Firstly, it can be averaged and thus used to set the frequency of the activity, and secondly, it can influence the motoneuronal activity underlying a particular phase of the activity (Wilson & Gettrup, 1963; Wilson & Wyman, 1965; Davis, 1968*b*, 1969*a*,*b*, *c*; Pearson, 1972; Kater & Rowell, 1973; Spirito, Evoy & Fourtner, 1973).

It is becoming generally recognized that phasic information from proprioceptors is important in controlling a particular phase of an activity in systems where there is a high probability of cycle to cycle fluctuations in load. This generalization has been formulated in systems where increased load results in increased output along the compensating motor pathway (Davis, 1969*a*; Pearson, 1972; Kater & Rowell, 1973; Spirito, Evoy & Fourtner, 1973).

Changes in load could result in changes in muscle tension, extra-muscular tissue or joint stresses, or in the rate of tissue or joint movement, or a combination of these factors. Receptors registering such changes have been shown to be responsible for the increases in motoneuronal burst lengths or intra-burst frequency which have been assumed to compensate for the changes in load. Within the antennule system, however, joint flexion is necessary for the motoneuronal activity required for the extension phase of a flick (Fig. 28). In this system increased load would thus result in a decrease of the flexion movements and thus an abolition of extensor activity. It should be noted that a feature of this system is that increased load does not facilitate output along the compensating motor pathway (see Fig. 28).

In any medium where the resistance to repetitious fast movements is likely to fluctuate, reflex excitation of related movements by joint movement receptors will, in a mechanistic sense, appear as a rather inefficient system. This statement, however, assumes that it is the sequence of related movements that is required, rather than the function which they might serve under stable conditions. The function of the flexion phase of flicking is probably to circulate water around the chemoreceptive aesthetasc setae (Section 2 *(a)*, Chapter IV). The antennules are often rotated so that flicks are directed into existing water currents (Section 2 *(d)*, Chapter III). Under these conditions there is probably reduced flexion at the DS-OF and particularly the MS-DS joints but no reduction in the circulation of water around the aesthetasc setae. Reflex control of extensor motoneuron A30F would thus be useful in ensuring that extension at the MS-DS joint only occurs during flicks which resulted in appreciable flexion at this joint.

A second most attractive possibility stems from the observation that motoneuron A30F is not active during flicking when the MS-DS joint is being held in a flexed position by activity in slow motoneuron A31S (Section 3 *(d)*, Chapter III). This might be expected, as in this posture there is little flexion at the MS-DS joint during flicking. The absence of flexion probably arises from the orientation of fast muscle 31F being such that the amount of flexion it can produce is reduced when the MS-DS joint is partially flexed. In addition, joint resistance to flexion probably increases with the degree of initial flexion. The antennules are held flexed at the MS-DS joint during flicking in a crab that is withdrawn into its shell. In this state extension at the MS-DS joint would result in the outer flagellum hitting the inside of the shell. If this did occur, motoneuron A31S and possibly A31F-S would be excited and tonic flexion would be reestablished at the MS-DS joint (Section 3 *(c)*, Chapter III).

It seems likely that the reflex control of extension during a flick can be tolerated because circulation of water around the aesthetasc setae may be achieved in spite of considerable modification of the component movements of a flick. The contribution of reflex control to flicking can thus be regarded as complementary to the general concept that cycle to cycle load fluctuations are usually correlated with a greater utilization of phasic feedback information in forming the motor output (Pearson, 1973; Kater & Rowell, 1973).

V. SUMMARY

The surface structures of the antennular flagella of *Pagurus alaskensis* (Benedict) are described in detail. Attention is directed towards the surface morphology of possible sensilla. These may be divided into two major categories: (1) exoskeletal pores (1·0 to 3·0 µm in diameter) and (2) a variety of types of setae. In addition, small (0·1 to 0·2 µm) pits occur in the exoskeleton which are not considered to be sensory in function. The exoskeletal pores are found at fairly specific locations on both the inner and outer flagella. This is particularly true of their distribution on the short segments of the outer flagella. Neither the inner nor the outer flagella are bilaterally symmetrical with respect to their setal armature. On the outer flagellum six groups of setae may be distinguished: the lateral-mesial, the dorsal, the ventral, the accessory, the aesthetascs, and the setae of the distal segment. On the inner flagellum two groups of setae may be distinguished: those of the mesial and lateral rows. The morphology orientation and location of all the flagellar setae are defined, and where possible the numbers of the various morphological types within the specific setal groups are given. Many setal types have obvious apical pores and yet no pores could be found in the chemoreceptive aesthetasc setae. The functions of the various setae are discussed in relation to their topographical position and to existing electrophysiological and behavioural data. Some suggestions are made in regard to future experiments aimed at determining the whole-animal significance and central connections of specific sensilla or groups of sensilla.

The antennular activities of *P. alaskensis* were studied with the aid of motion pictures taken at speeds of 50, 200 and 400 frames/sec. Most movements of the antennule represent one of four types of antennular activity: flicking, rotation, wiping and withdrawal. These activities are described in detail. Water resistance forces contribute to the timing and duration of some antennular movements. Flicking occurs non-rhythmically and flicks of the left and right antennules are never synchronized. The factors which influence the mean frequency of flicking are discussed. The timing of joint movements during a flick, and the morphology of the outer flagellum and the aesthetasc setae, appear to be adapted to facilitate splaying of the aesthetascs. It is proposed that this splaying might facilitate the chemoreceptive process by circulating water around the aesthetasc setae. During antennular wiping the endopodites of the 3rd maxillipeds are used to remove debris caught amongst the aesthetasc setae. Light mechanical stimulation of the aesthetascs is probably sufficient to elicit wiping. The antennules are reflexively withdrawn from certain stimuli either by extension or by slow or fast flexion. The functional significance of the withdrawal reflexes are discussed in relation to the stimuli involved and the form of the reflexes. Continued application of certain stimuli to the antennules, eyestalks, antennae or body results in tonic flexion withdrawal which involves postural modifications of the antennules, eyestalks and antennae.

The motor innervation and musculature of the medial and distal segments of the antennule have been described anatomically. Intracellular recordings within these muscles and simultaneous monitoring of whole-muscle tension have been used to define the motoneurons and contractile properties of the muscle fibres they innervate. The motor system consists of two fast, two slow and one mixed muscle which are innervated by seven motoneurons (A30F, A30S, A31F, A31S, A32F, A32S, A31F-S). No evidence of postsynaptic inhibition was found. The motor innervation is such that this system may be divided into three components: a phasic component (motor units 30F, 31F and 32F), a tonic component (motor units 30S, 31S and 32S) and a phaso-tonic component (motor unit 31F-S). The tonic component is adapted to produce fine tonic tension in response to relatively low-frequency (5 to 10/sec) motoneuron discharge. It is suggested that this may be important for the postural control of appendages which, owing to the density of the environmental medium, are relatively weightless.

Using electromyogram recording from the antennular muscles of the medial and distal segments, the patterns of activity in specific antennular motoneurons have been described during antennular flicking and antennular withdrawal. Only the phasic component of the antennular motor system, motoneurons A30F, A31F and A32F, is active during antennular flicking. The flexion phase of a flick is the result of a burst of variable duration and number of spikes within flexor motoneurons A31F and A32F. In any animal the burst in motoneuron A31F precedes the burst in motoneuron A32F by a relatively constant duration in the range of 1·8 to 2·1 msec. The functional significance of the flexor patterns are discussed with reference to a previously proposed function of flicking. The extension phase of a flick is the result of a burst of variable duration and number of spikes in extensor motoneuron A30F.

The first spike in motoneuron A30F follows the first spike in motoneuron A31F by a delay of 20 to 40 msec. During tonic flexion at the medial segment-distal segment joint there was frequently no activity in motoneuron A30F during antennular flicking. Extension-withdrawal and slow flexion-withdrawal reflexes, tonic flexion withdrawal and maintained flexion at the medial segment-distal segment joint result from activity in the tonic component of the antennular motor system: motoneurons A30S, A31S and A32S. The patterning of activity in these motoneurons is discussed. Fast flexion-withdrawal reflexes result from a burst of spikes in motoneuron A31F-S which constitutes the phaso-tonic component of the antennular motor system. During high-frequency activity (15 to 60/sec), reciprocity exists between the slow flexor motoneurons A31S and A32S and slow extensor motoneuron A30S. This reciprocity appears to result from inputs which excite the flexor motoneurons while inhibiting the extensor motoneuron and inputs which excite the extensor motoneuron while inhibiting the flexor motoneurons.

The effects of altering sensory input on the motoneuronal patterns underlying antennular flicking have been tested. Removal of the short segments of the outer flagellum, in most animals, results in a marked reduction of bursts of more than 2 spikes in flexor motoneurons A31F and A32F. During a flick the timing between the burst in motorneuron A31F and the burst in motoneuron A32F is insensitive to alteration of sensory input. During flicking, flexion at the joints between the medial and distal segment and the distal segment and outer flagellum is usually necessary for the consistent excitation of extensor motoneuron A30F. Threshold stimulation of flexor muscle 31F is only occasionally sufficient to excite motoneuron A31F in 40% of the animals tested. This stimulus is ineffective in exciting motoneuron A30F following immobilization of the medial segment-distal segment joint. The results are incorporated into a model in which flexor activity is elicited by activation of a trigger system, while extensor activity is reflexively elicited by feedback from phasic, unidirectional receptors sensitive to joint flexion. The functional significance of reflex control of extensor activity is discussed in relation to both the form and proposed function of antennular flicking and to the general role of reflexes in stereotyped activities of other invertebrates.

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